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THE LIFE CYCLE OF MACROSIPHUM SOLANIFOLII WITH SPECIAL REFERENCE TO THE GENETICS OF COLOR

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THE large aphid of the potato and the rose, *Macrosiphum solanifolii,* exists in two color varieties, green and pink, respectively. The precise relation of these varieties to each other has hitherto been unknown. Patch (1915) reared 500 progeny, all pink, from nine original pink parents, but in correspondence with other entomologists it is sometimes stated that the species "throws sometimes green, sometimes pink" individuals, as if there were a suspicion that one or both of the color types might produce both kinds of offspring. Certain facts related below could easily lead a casual observer to think that at least the green variety sometimes produces the pink, and it is not unlikely that the idea has been entertained that either variety may produce both. There is nowhere in the literature, so far as I can find, any information concerning the relations of the two varieties through sexual reproduction. The work described in this paper was undertaken to ascertain the facts on these points.

The species exists in eight different forms, four in the green variety and four in the pink. Thus, there are apter-

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ous parthenogenetic and alate parthenogenetic females, oviparous (sexual) females and males in each color variety.

DESCRIPTION OF THE EIGHT FORMS

The more striking structural characters are the same in corresponding forms in both color varieties, and are given here for the guidance of those who may wish to study the species. The apterous parthenogenetic females have 4 to 6 sensoria on the third segment of each antenna, no sensoria on the hind tibiae and have no ocelli. The alate parthenogenetic females have three ocelli, 15 to 18 sensoria on the antennae and no sensoria on the hind tibiae. Oviparous females are wingless, have 4 to 8 sensoria on the antennae and hundreds of sensoria on the hind tibiae, which are swollen and brown. Males, which are winged, have 25 to 40 sensoria on the antennae and none on the hind tibiae.

The physical basis of the color in each of the eight forms is briefly described in the following paragraphs.

Pink Apterous Parthenogenetic Female: The pink color of this form is due to minute spherules of a pink or red color contained in the body fluid. The largest of these spherules are about 1 μ in diameter, and the smallest presumably below the limits of visibility. There are hundreds of thousands of them in a single aphid. Being in the body fluid, these spherules are crowded aside by the organs or definite tissues, or by larger bodies of other kinds contained in the body fluid. The color pattern is therefore somewhat variable in different individuals because it is determined in part by the distribution of the internal organs, including the passages filled with unborn young, in part also by inequalities in the distribution of the red spherules in spaces which they might occupy.

Very young embryos while still in the body of the mother do not possess any of the red spherules and are colorless; but the red substance appears before birth. A color pattern is usually more definite in the young than in

the adult. There is, in these young insects, a colorless strip along the middle of the dorsum of the abdomen, due to the heart and principal vessel, and a segmental arrangement of the color on both sides of this strip. About seven blocks of red on each side of the middle line are separated from each other by colorless strips. The thorax and head of the young aphid are of a paler red color, due to fewer red spherules.

Pink Alate Parthenogenetic Female: The pink color is due to red spherules of the same sort as those in the apterous female. For the most part they are no larger than in the apterous female; but in some specimens a few larger globules, up to 2 or 3 μ in diameter and of a reddish orange color, have been found. These larger orange globules may be of a different substance from the smaller red ones.

The distribution of these red spherules differs from that in the apterous form in certain respects. Thus, there are few of them in the meso- and metathorax, owing to the great development of the muscles there, so that this region is yellowish. Also there is not infrequently a honeycomb arrangement of the red spherules in the dorsal region of the abdomen, which is due to the presence of larger globules of a colorless or faintly yellowish substance thought to resemble fat, in the body fluid, with the red spherules crowded between them and collected on their surfaces.

Pink Oviparous (Sexual) Female: The color of the pink oviparous female is due primarily to red spherules not different from those of the parthenogenetic female. These spherules are, however, usually distinctly more abundant, so that the oviparous females are more opaque. This opacity is observable frequently in the early nymphal stages, so that the sexual females can be recognized before the distinctive structural features appear. In addition to the red spherules there are often numerous globules of larger size, up to 5 or 6 μ, of a bright yellow color, which no doubt help to render the

aphid more opaque than the parthenogenetic females, without modifying to any considerable extent the general red color.

The eggs of the pink oviparous female are bright green in the body and when first laid, but turn black later. Accumulation of eggs in the female's body displaces the red spherules and gives the whole insect at first a paler pink, then a greenish appearance.

Pink Male: The basis of color in the male is more variable than in any of the females. The body fluid contains large numbers of globules of varying size and color in different individuals. In one specimen they were found to range from the smallest visible size up to 13 μ; in another from 1 to 20 μ; in a third 4 to 10 μ; while in another none were larger than 3.5 μ. In one individual all globules up to 4 μ in diameter were colorless or of a pale yellow. In another, some globules were colorless, some yellow, some orange, some reddish orange, and these colors were found in globules of all sizes. Another adult had only pale yellow globules. The fact that the chitinous exoskeleton is brown on the thorax makes these variations in the internal color less conspicuous than they would be in any of the females, whose chitin is colorless over the entire body.

Green Apterous Parthenogenetic Female: Color in this form is due to minute green spherules, from 1.5 μ downward in size, contained in the body fluid. They are almost, or quite, as numerous as the red spherules of the pink apterous female, but the maximum size is a little greater. The color pattern is dependent on the distribution not only of the spherules, but of the internal organs, just as in the pink variety. Larger globules of fat (?) were uncommon in the green apterous parthenogenetic female, so that the general color is almost solely that of the green spherules.

Green Alate Parthenogenetic Female: In the winged parthenogenetic females the externally visible color is usually slightly yellowish green. The green color is due

to many thousands of minute spherules similar to those of the apterous female described above. In addition to these are thousands of larger globules, up to 7 or 8 μ in the individuals examined, some of which are of a bright yellow color, others faintly yellow or colorless.

Green Oviparous (Sexual) Female: The young oviparous females of the green variety are very pale green. As they approach maturity they usually become yellowish, and at the height of their egg-laying period are commonly of a bright yellow. The color is found in multitudes of globules in the body fluid, ranging in size from barely visible up to 20 or 30 μ. In young individuals comparatively few of these are yellow, the rest colorless. In older females practically all the globules of a diameter of 4 or 5 μ or less are bright yellow, while the larger ones are nearly colorless. The increase in depth of yellow with age is due to the assumption of the yellow color by a larger number of the globules of nearly all sizes.

The newly laid eggs of this oviparous female are bright green, turning black later, as in the pink variety.

Green Male: This form is better styled the male of the green variety, since at no stage is it really green. The young male is either of a pink, dull pink or grayish or pale ocher color. The adult is internally yellowish or salmon colored, with brown chitin on the thorax, which modifies the general appearance. The color is due to hosts of globules in the body fluid, ranging from a minimum diameter of about 1.5 μ up to 3 or 4 μ in some individuals, or to 8 or 9 μ in others. In the pink young individuals, some of these globules are of a bright orange pink color, some colorless, with all gradations of color between these extremes. The brightest colors in the "green" male are brighter than the red of the spherules of the pink variety, but there are fewer of them, hence the duller pink color. The adult male contains usually a few orange spherules, some of paler yellow or salmon color, while probably about 80 or 85 per cent. are colorless. Apparently none of them are green.

Although the larger globules mentioned above in the descriptions of colors are tentatively regarded as composed of fat, or a fat-like substance, there is no certainty as to their composition. Whether there are two or more classes of substances included in the spherules is unknown, and the nature and source of the colors in them is likewise conjectural. These questions are under investigation.

DIFFERENCES AMONG PARTHENOGENETIC LINES

The outstanding distinction between parthenogenetic lines in this species is that between the pink and the green variety. Each breeds true to its own color by parthenogenetic reproduction. Over ninety thousand individuals have been reared in my experiments, and there has not been one exception to the rule that pink parthenogenetic females produce only pink offspring, and green parthenogenetic females produce only members of the green variety. This confirms the results of Patch (1915) referred to above and removes any suspicion that the color difference is individual rather than varietal. The idea that parthenogenetic females of either color might produce offspring of either or both colors, if it existed, may have been due to the fact that individuals of the two colors live side by side on the same plant, that the males of the green variety are pink or nearly so in their young stages, and that, as described below, certain green parthenogenetic lines may have reddish-orange oviparous females.

Other differences between the pink and green varieties occur. Pink lines have always employed a longer time in parthenogenetic reproduction before entering the sexual phase than have the green lines. As a result the sexual period of the green variety is usually well advanced before that of the pink variety begins. One season the difference was so great that there was no overlapping of the sexual periods of the two varieties. Perhaps associated with this difference there is a difference in the rate of

reproduction. In general, green individuals produce more offspring per day than do pink ones. By removing apterous pink and green aphids from plant to plant each day and ascertaining the number of offspring produced in each preceding 24-hour period, it was ascertained that the average number of offspring per day among the green females tested was 3.81 ± .073, while the mean for the pink females was 3.30 ± .064. These figures were obtained during a period of moderately warm weather one summer, and with the parthenogenetic lines then being used. They probably would not represent an annual average, nor an average of all lines, but do represent a difference between pink and green lines, which casual observation indicates is rather general.

Within the pink variety there are differences among lines, in the intensity and quality of the red color, due to variations in the number of colored spherules contained in the body fluid and to the nature of the color borne by them. Brick red, ocher red, orange vinaceous and flesn pink (Ridgway's *Color Standards and Nomenclature*) have been observed in different parthenogenetic lines.

Variations within the green variety are not conspicuous, so far as the parthenogenetic females are concerned, the extremes of color in the apterous individuals in the lines I have observed being dull green yellow and courge green (Ridgway). One much more striking variation was a green line in which the adult sexual females were, on part of the body, of a salmon-orange color, the rest of the body green. The color of the adult sexual female in most green lines is wax yellow. The parthenogenetic females of this line were not noticeably different from those of other green lines. Mottled pink and green individuals are mentioned by Patch (1915) as occurring late in the season. It is uncertain whether these were of the sort here described, which were obviously mottled owing to differences in the color and distribution of the contained globules, or whether they were green females containing pink male embryos or pink oviparous females containing

green eggs. Any of these types should appear late in the season.

VARIATIONS WITHIN PARTHENOGENETIC LINES

Pink parthenogenetic lines have usually, in my experiments, become somewhat paler with age. The recorded color of one line when first collected in the spring was ocher red but in late summer was classed as orange vinaceous. Practically all lines varied in the same way, so that differences between lines observed in the spring were not diminished by the general fading of the color. Whether the fading is due to environment, such as a change in the quality of the food plant, or to something inherent in the animals themselves, is not known.

An extreme case of color change was observed in one pink apterous female which, when young, was distinctly pink, though somewhat paler than its sisters, and which later came to be of a translucent green. The genetics of such a change seemed more important than the physical basis of the color, so the female was kept as long as there seemed to be any chance that it would reproduce. It did not reproduce, however, and finally disappeared from the plant, presumably having died and dropped to the soil of the pot, where it could not be found. The nature of the color change is therefore unknown, but the failure to reproduce suggests that the individual was diseased.

INHERITANCE OF COLOR IN CROSSES

Matings were attempted in all possible ways in order to test the inheritance of color. These matings were made in some instances between males and sexual females of the same parthenogenetic line, in which case they may be spoken of as inbred matings. In other cases different green lines were mated with one another, different pink lines with one another, or pink lines with green ones.

In making matings, sexual females and males were confined on the same plant under a lantern globe, and the females, in case copulation occurred, subsequently laid

their eggs on the stem or leaves of that plant. When the plant began to die those parts which bore the fertilized eggs were preserved. The eggs were given varying treatments, none of which resulted in more than a very low percentage of hatching. The first season in which fertilized eggs were obtained (1916) the remains of the plant with eggs on it were laid on the soil of a pot beside a young plant in the greenhouse. Only one egg in this lot hatched, requiring about two months in the egg stage. In one later season the plants with eggs on them were preserved dry for several months and then placed on soil by a growing plant; none of these eggs hatched. Another method was to place the plants with attached eggs in glass tubes, closed at the ends with gauze, and expose them for several weeks or months to the rigors of winter, under leaves and snow, after which they were brought to greenhouse conditions. Even with this treatment, which would appear to be nearly normal for the species in nature, the percentage of hatching was very low, many lines not producing a single viable egg. No single method in which the eggs were kept somewhat moist seemed to be particularly better than other methods which maintained moisture.

In all cases, a group of eggs placed beside a young plant to hatch was left there only several days and then moved to a new plant. No immediate examination of the plant was made, since it is extremely difficult always to discover a single young aphid on a potato plant with its hairy, curling leaves. Instead, the plant was kept several weeks, at which time many aphids would be present if any of the eggs had hatched.[2] This method made it possible that more than one egg hatched in the two- or three-day period during which the eggs remained beside one plant. It is not likely that this occurred very often, for

[2] It is to be regretted that this method of obtaining aphids from fertilized eggs was not calculated to reveal the stem mothers. Although these aphids have apparently been obtained from fertilized eggs by earlier investigators, there does not exist, so far as I can find, a satisfactory description of the female so produced.

TABLE 1

SUMMARY OF MATINGS BETWEEN MALES AND SEXUAL FEMALES OF THE SAME GREEN LINES OF MACROSIPHUM SOLANIFOLIA RESULTING IN THE PRODUCTION OF FERTILIZED EGGS, AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1916	Potato	78	1	0	Potato
1917	Potato	276	0	0	.....
1919	Rose	284	0	0	.....
		461	0	0	.....
		326	2	0	Potato
1920	Rose	641	0	0	.....
		382	0	0	.....
		567	4	0	Potato
		448	2	0	Potato
		490	0	0	.....
1921	Rose	183	0	0	.....
		265	2	0	Potato
		417	0	0	.....
		314	2	1	Potato
		482	0	0	.....
		334	3	0	Potato
		318	0	0	.....
		427	0	0	.....
		366	0	0	.....
		282	4	0	Potato
		165	0	0	.....
		325	0	0	.....
		229	0	0	.....
		403	0	0	.....
1922	Rose	227	0	0	.....
		84	2	0	Potato
		177	0	0	.....
		351	1	0	Potato
		272	0	0	.....
		55	0	0	.....
		475	0	0	.....
		269	3	1	Potato
1923	Rose	401	0	0	.....
		762	4	0	Potato
		363	0	0	.....
		499	0	0	.....
		554	2	0	Potato
Total		12952	32	2	

the eggs were hatching so infrequently that only once in all the experiments did two successive plants used for one batch of eggs have aphids on them, and in no case were both pink and green aphids on one plant. Large numbers of plants could be used in this way without entailing prohibitive labor.

It is important to know whether covering the plants with lantern globes with gauze tops excludes foreign aphids. Macrosiphum is not one of the excessively restless species, and there is no internal evidence in the very long experiments testing the distinctness of the pink and green varieties that any contamination occurred. Nevertheless, numerous direct tests of the efficacy of isolation by lantern globes were made by covering plants having no aphids on them and setting them near infested plants. In no case did the aphids reach any of the plants thus guarded.

As was pointed out above, the percentage of hatching eggs was always small. Nevertheless, enough offspring have been obtained to indicate clearly the genetic nature of the color differences. The matings, which extended over the period from 1916 to 1923, are collected in tables 1 to 5, classified with respect to the type of mating. All information that is thought to bear on the questions discussed in this paper is given in these tables.

Attention is called to the fact that crosses between green and pink lines were made only with green females and pink males. This is owing to the fact, stated above, that the pink variety entered its sexual phase several weeks later than the green variety. In practically all lines the males appear before the sexual females, so that by the time sexual females of the pink lines were produced the males of the green lines were gone. Usually there were green line females when pink males appeared, and it was with these that the crosses were effected.

The twelve F_1 lines derived from the eggs in Table 5 were preserved for a time, as were also the two green lines from pink parents recorded in Table 4, in an at-

TABLE 2

SUMMARY OF MATINGS BETWEEN MALES AND SEXUAL FEMALES OF SUPPOSEDLY DIFFERENT PARTHENOGENETIC GREEN LINES OF MACROSIPHUM SOLANIFOLIA, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS, AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them.		First food plant of offspring
			Green	Pink	
1921	Rose	284	2	0	Potato
		424	0	0	.....
		195	2	1	Potato
1922	Rose	782	1	0	Potato
		156	3	1	Potato
		445	0	0	.....
		722	0	0	.....
1923	Rose	557	1	0	Rose
		335	0	0	.....
		681	2	1	Rose
		595	4	0	Rose
Total		5176	15	3	

tempt to secure further generations of parthenogenetic lines from them. Seven of the fourteen, including the two from pink parents, were lost in a period of depression in the winter of 1923, due probably to unhealthy food plants (potato). The four produced in 1922 (Table 5) went into the sexual phase the following summer, and their fertilized eggs were kept in moist air at ordinary temperatures, but none hatched. The remaining three lines from eggs produced in 1923 went into the sexual phase in February and March, 1924. Their eggs were at once subjected to moderately low temperatures out of doors and then brought into the greenhouse, but none hatched. This failure to hatch removes one important element in determining the mode of inheritance of color, and makes the explanation rest upon the data obtained up to the late fall of 1923. Fortunately, however, there can be little doubt regarding the general nature of the genetics of color.

TABLE 3

SUMMARY OF MATINGS BETWEEN MALES AND FEMALES OF THE SAME PINK PARTHENOGENETIC LINES OF MACROSIPHUM SOLANIFOLIA, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1921	Rose	37	0	0	.....
		97	0	0	.....
		22	0	0	.....
		104	0	0	.....
		34	0	0	.....
1922	Rose	188	0	0	.....
		65	0	0	.....
1923	Rose	419	0	1	Rose
		289	0	0	.....
		58	0	0	.....
		456	0	0	.....
Total		1769	0	1	

The explanation of the inheritance of color remained uncertain until the results from the matings of 1923 were obtained. From the first, inbred or crossbred lines produced mostly green individuals, but occasionally (beginning 1921) a pink one. The crosses between the two color varieties have yielded only green lines. The first hatchable egg of a pink line (obtained in 1923, Table 3) yielded a pink individual. All the foregoing results could have been explained by assuming a single factor difference between the color varieties, with green dominant. However, in the 1923 results were two cases (Table 4) in which crossbred pink lines gave green offspring. To explain all these results it seems necessary to assume that color is dependent upon multiple factors; and that while the factors possessed by the pink line are largely those producing pink color, it is nevertheless possible to obtain from the pink lines (at least by crossing different lines) the right combination of factors for green color. This

assumption is moreover in better keeping with the variability of color exhibited by the various lines, especially in the pink variety.

If the conclusion that multiple factors are involved in the production of color is correct, further progress will probably require a prohibitive amount of labor, unless some means is found of inducing a larger proportion of fertilized eggs to hatch. The next desirable steps would be to ascertain how many factors are concerned, whether they are of one kind or several, and possible differences in the relations of factors to one another. Thousands of hatchable eggs might be required to attain these ends. Whether an increase in the hatchability of eggs may be artificially effected is uncertain, since there is no necessity for concluding that more of the eggs hatch even in nature. In a closely related species, *Macrosiphum granarium,* Phillips (1915) has found a similar high mortality of the fertilized eggs, which may be a normal condition in this genus and present insurmountable obstacles to further work.

RATE OF REPRODUCTION PROBABLY NOT INDEPENDENTLY INHERITED

As previously stated, the green lines tested produced a larger mean number of offspring per day than did the pink lines being reared at the same time, and casual observation leads me to believe that this is a general difference. Inheritance of this difference could not be studied in a wholly satisfactory manner, since in every case, by the time the F_1 line appeared, the green parent line was no longer in existence because it had gone completely into the sexual phase. No direct comparison with the green parent could therefore be made. In the case of the eight F_1 lines derived from crosses in 1923 in Table 5, the pink parent line from which all these were descended was still alive when the F_1 green lines started. Unfortunately, five of these lines were lost before the rate of reproduction was determined. The three remain-

TABLE 4

SUMMARY OF MATINGS BETWEEN MALES AND FEMALES OF SUPPOSEDLY DIFFERENT PINK PARTHENOGENETIC LINES OF MACROSIPHUM SOLANIFOLII, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS AND THE OFFSPRING FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1923	Rose	286	0	1	Rose
		394	0	0	.....
		427	1	1	Rose
		580	1	1	Rose
Total		1687	2	3	

ing ones gave, at that time of year (mid-winter), daily output of offspring as follows: 2.82 ± .088, 2.91 ± .090, and 2.95 ± .094. The daily output of the pink parent line, then seven months old, at the same time was 1.53 ± .075.

A more important comparison may be made in the case of the two green lines derived from pink parents in Table 4, whose rate of reproduction was determined before the lines were lost. These two lines produced offspring at the daily rate of 2.56 ± .061 and 2.87 ± .072, respectively. The particular pink lines from which these were descended had been discarded to make room for other experiments, but the pink line mentioned in the preceding paragraph was being reared and was producing at this time, as stated above, 1.53 ± .075 offspring per day.

The difference between these two hybrid green lines and the (perhaps) unrelated pink line with which they were compared is greater than the difference between green and pink lines given earlier in this paper. Possibly this greater difference is due to the fact that the lines were not of the same age when compared. The hybrid green lines were about one month old, the pink one about seven months old. Whether age of the line or

TABLE 5

SUMMARY OF MATINGS BETWEEN MALES OF PINK LINES AND FEMALES OF GREEN LINES OF MACROSIPHUM SOLANIFOLII, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1922	Rose	75	0	0	.....
		126	0	0	.....
		88	1	0	Potato
		255	3	0	Potato
		66	0	0	.....
1923	Rose	879	4	0	Rose
		1063	4	0	Rose
Total		2552	12	0	

long-continued uniform conditions of life could cause such a difference is unknown.

Most of the other F_1 lines were reared through a number of generations to test their uniformity of color, but were discarded, before the rate of reproduction could be ascertained, on account of the labor involved.

ORIGIN OF PINK LINES IN NATURE

That pink lines can be derived from green parents or from pink parents, under greenhouse conditions, has been demonstrated. Whether they may also be descended from one green and one pink parent has not been shown, though such a result would seem not improbable. In nature it is questionable whether all these possible sources of pink lines are ever available in our latitude. The parthenogenetic phase of the pink lines lasts so long that it is doubtful whether the summer season in this part of the world will suffice for a well-marked sexual phase, unless in exceptionally late autumns. Under these circumstances the pink lines of each year must be derived

wholly or in part from the green lines of the preceding season.

Species Relation of the Two Varieties

Probably no systematic zoologist would entertain a proposal to place two color varieties of any insect in different species, if the chitinous structures of both were identical as they are in *Macrosiphum solanifolii,* whether he knew anything of the genetics of color or not. A geneticist, however, might be inclined to give them separate specific recognition if it could be shown that neither variety produced the other. Any one could have determined, with a little trouble, that during the parthenogenetic phase the two varieties are absolutely distinct. Whether they are thus distinct in bisexual reproduction was unknown until the work reported in this paper was done. Since, as here shown, either variety can produce the other, and under natural conditions the pink variety probably usually and perhaps always comes from the green variety, even a geneticist could not maintain that the varieties constitute different species.

Alternation of Food Plants

Macrosiphum solanifolii is very tolerant of its food, having been discovered on scores of different kinds of plant. It is obvious, however, to any one who has followed the species through its entire cycle that the great majority of plants which may properly be termed its hosts are tolerated only by the sexual female. The sexual female comes to rest on almost any plant on which it happens to be placed, and commonly remains long enough to be regarded as having "settled." Whether it will lay its eggs on most of these is unknown, but I have observed its eggs on several plants from which the parthenogenetic forms always departed immediately when placed on them. If, therefore, plants were designated as proper hosts only if all forms of the species lived upon them, the list would be greatly reduced. But even with this restriction, the

host plants of *Macrosiphum solanifolii* would be much more numerous than for the majority of aphid species; and the species has become a serious pest on plants as different as potatoes, kale, spinach and egg-plant.

Nevertheless, there is in the literature a tendency to describe this aphid as alternating between the rose and the potato. Part of this tendency is no doubt due to extending to all aphids a conception which is strikingly correct for certain species which are strictly limited to two kinds of food plant, but is partly justified by observations in nature. Patch (1915) refers to the rose and the potato as "two favorite food plants," but recognizes that the species is at home on many others. She finds, nevertheless, that the potato is the common summer host, that potato plants are deserted in late summer and that the rose is favored by the "fall migrants." In the spring the first individuals are found on the rose, but migration to potatoes occurs in early summer, and one infers from her description that the rose is free from them during the summer. Regan (1917) also describes a sort of alternation between host plants, but in very general terms.

Observations recorded in this paper indicate that whatever alternation between hosts occurs in this species has no significance; that is, it indicates nothing whatever concerning the inherent nature of the insects or their cycle. The stem mother, immediately upon hatching from the fertilized egg, feeds upon either the rose or the potato, whichever is available, as is shown in Tables 1 to 5. They continue to live on either plant so long as tender tissues are available on them, and may be transferred from either one to the other at any time the year round. There is little difference between winged and wingless females in this respect, for both commonly remain, without confinement, on a plant so long as conditions are favorable, and both migrate freely when conditions are unfavorable. The winged females presumably travel farther than wingless ones, but there is little to indicate that they are more successful in finding suitable new hosts except in cases where large regions are rendered uninhabitable. In par-

ticular, there is nothing to show that the migration from the rose to the potato is done only or even chiefly by the winged female. The sexual forms live with equal readiness on either plant, and have laid their eggs on potatoes, while Smith (1919) reports finding the eggs of this species on the rambler rose.

A rough alternation between potato and rose does, however, occur. My observations in this region support those of Dr. Patch in Maine. The species is nearly always to be found on roses earlier than on potatoes. During the summer it is usually absent from the rose, but regularly occurs on the potato at that time. I have found sexual forms on the potato in late summer or early autumn, but never on the rose. How or when the rose again becomes infested is uncertain. This approximate alternation, in time, between the rose and the potato does not always occur, for sometimes aphids may remain on the rose all summer. In 1922 they were found on the same stem of a certain rosebush continuously from May 18 to September 9. Winged individuals were usually present during that time, at least after the first several weeks, although it is not known that any one winged individual remained very long.

How, then, is the usual temporal alternation between the rose and the potato accounted for? It is probably true that young growing rose shoots are available earlier in the season than are potatoes, which may account for the earlier discovery of them on roses. Their disappearance from the rose in most years is, presumably, due to rain and wind. The aphids are easily jarred off their food plants. On the rose they live only on the stems, near the tips where the stem is still tender, or on the very young leaves, and never on the older leaves, which are thin and papery and would probably furnish little food. The tips of the stems are whipped about violently by strong wind, and are nearly always so located as to be beaten by raindrops. I have repeatedly observed that rose tips so thickly covered with aphids that there ap-

peared to be no room for any more were, immediately after a rain- or windstorm, completely or practically bare. The inimical effect of rain upon aphids has been noted by many writers (Patch, 1915; Phillips, 1916; Houser, Guyton and Lowry, 1917; Britton, 1918; and others). On potatoes, the aphids are not limited to the stems, but very often settle on the under surface of the leaves. The leaves are usually concave downward, which probably affords considerable protection from rain. Moreover, the potato plant is hairy, which may make it more difficult to dislodge the aphids. The aphids do collect in great numbers on the tender growing stems of potatoes, just as on the rose, and I have observed that a heavy rain or wind may largely dislodge them from such places; but there are usually individuals under the leaves of such plants by which the whole plant may soon again be stocked.

That the disappearance of the aphids from the rose in summer is due to the mechanical agency of rain and wind seems to be confirmed by the weather record of the one season, 1922, in which I found them on the rose all summer. The summer of 1922 was unusually dry. The total rainfall in Ann Arbor during the five months, May to September, was only 10.18 inches, as compared with 16.37, 17.72, and 18.31 inches for three other recent years. The maximum daily rainfall is of interest in this connection. The last rain of any importance in the spring of 1922 was on May 19, 1.38 inches, probably before stem mothers had ceased to hatch from overwintering eggs. The largest single rain in June was on the 10th, 0.66 inch; in July, on the 12th, 0.38 inch; and in August, on the 29th, 0.63 inch. The first considerable rain in the fall was on September 11, 1.38 inches. September 9 was the last day on which aphids were found on the rose; no further observations were made until just after the rain of the 11th, and then the aphids were all gone.

SUMMARY

(1) *Macrosiphum solanifolii* exists in two varieties, pink and green, whose color is due to globules of different

colors and sizes contained in the body fluid. The several forms belonging to each of these varieties are briefly described.

(2) Pink lines differ from one another somewhat in color and green lines differ from one another, owing to differences in the color and number of globules contained in the body fluid.

(3) The pink color of a line commonly fades somewhat during the season; the cause of the change is unknown.

(4) In green lines the sexual (oviparous) female is most often wax yellow in color, sometimes greenish; in one line, however, the sexual females were mottled with salmon-orange on the abdomen.

(5) The sexual phase usually appears later in the season in pink lines than in green lines.

(6) Pink parthenogenetic females produce only pink offspring. Green parthenogenetic females produce only members of the green variety, some of which, however, are not green.

(7) Very few of the fertilized eggs of sexual females hatch. No treatment capable of increasing their viability has been discovered.

(8) Sexual females of a green line mated with males of the same line produced mostly green offspring, but two of them were pink.

(9) Sexual females of one green line mated with males of a supposedly different green line produced offspring that were mostly green, but several pink ones were included.

(10) Pink sexual females mated with males of the same line produced only pink offspring. Since there were only two of these, it is thought that green offspring might also be obtained from such parents.

(11) Pink sexual females mated with males from other pink lines produced both green and pink offspring.

(12) Sexual females of green lines mated with males of pink lines produced only green offspring, though it is thought that pink offspring may occasionally be produced by such parents.

(13) The results of the above matings appear to indicate that color in these aphids is due to multiple factors. This assumption also fits the fact that lines differ among themselves in color, even within one color variety.

(14) The pink lines studied produced somewhat fewer young per day than did the green lines.

(15) This difference in the rate of reproduction exhibited by the two varieties seems to be associated with the color, and not to be independently inherited.

(16) Under natural conditions the pink variety probably descends chiefly, if not wholly, from the green variety each year.

(17) The approximate alternation of this species between the potato and rose is probably due to the earlier growth of shoots of the rose than of the potato and to removal of the insects from the rose by rain and wind, and does not indicate any inherent property of the aphids or of their cycle. Any form belonging to the species may live and reproduce on either of the principal host plants at any time of year.

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THE FACTORS OF INHERITANCE AND PARENTAGE AS AFFECTING THE RATIO OF ALATE TO APTEROUS INDIVIDUALS IN APHIDS[1]

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THE influence of parentage on aphid forms has been noted by Shull (1918), is very apparent from the earlier results obtained by Gregory (1917) and has been recently confirmed by Wadley (1923). The results of all three of these workers appear to be entirely in accord in two respects, *i.e.*, in regard to the existence of a tendency for winged parents to produce wingless offspring and for wingless parents to produce winged offspring. Shull also noted that winged males were the progeny of apterous mothers and that wingless oviparous females were the offspring of alate females. These observations caused him to relate the phenomena with Riddle's theory of sex. He regarded the winged agamic individuals and the males as the forms of high metabolism and low energy content and the wingless agamic females and the oviparous females as forms of low metabolism and high energy content.

Miss Gregory (Gregory, 1917), whose results were published a year earlier than those of Shull, came to the conclusion that the primary factor in determining the development of wings was the food supply. Her work was done with the green pea-aphid *Microsiphum destructor*. In one series of experiments, "G series" (Gregory, 1917, p. 302, table IV), it was found that winged parents even when starved produced no winged offspring.

Wadley observed the effects of various factors on progeny of different parentage and grandparentage. His

[1] Contribution from the Department of Zoology and Entomology, Iowa State College.

work in the main confirms that of Gregory and of Shull in regard to this tendency of parents to produce a higher percentage of offspring of the reverse form. In regard to the effect of wingless mothers on their progeny Wadley's results are not so confirmatory.

Previous to the work of Shull and of Miss Gregory the present writer (Ewing, 1916) had shown that temperature was an important factor affecting wing production in the apple-grain aphid, then known as *Aphis avenae* Fab. This work on temperature was only very briefly reported in my larger paper (Ewing, 1916) dealing with the inheritance and biology of the species. Even in a later paper (Ewing, 1917) little was added in regard to the results obtained pertaining to general biology.

Here further results are presented in which certain data and conclusions are given in regard to the effect of inheritance and parentage on the ratio of alate to apterous forms. Also in this paper an attempted explanation is given in regard to the phenomenon of parental and ancestral effect on aphid forms which for many years I have had in mind but which I have hesitated to give for lack of sufficient support by the way of experimental data. The recent publication of Wadley's excellent and comprehensive paper (1923) dealing with the identical species with which I worked has given so much of the desired data that it appears profitable to give this explanation.

The explanation is: It appears that in the case of the apple-grain aphid, and presumably in similar species of aphids, there is a type of inheritance not dependent upon the germplasm (or at least the chromosomes) but which modifies the expression of somatic characters which are dependent upon the germinal elements in the following important ways: (a) through the general metabolism of the individual which in turn is affected by its environment; (b) in such a way as to produce cumulative and hold-over effects from generation to generation; (c) through cumulative effects to produce an unbalanced state of metabolism which eventually rights itself by re-

turning towards the mean of the race. The term physiological inheritance might be applied to this parental and ancestral transmission of cumulative and oscillating metabolic inertia.

The Effect of Temperature

In 1916 (Ewing, 1916) the effects of temperature on the production of alate or apterous individuals in the apple-grain aphid, then known as *Aphis avenae* Fab., was shown. Lots of 10 each of reproducing wingless parent individuals were placed at different times for two days in a temperature cell at the following constant temperatures: 60° F, 70° F, 80° F, 90° F. At the end of two days these parents were removed and their progeny, thus affected, on the average for a day before their birth at the constant temperature at which they were born, were allowed to grow at that temperature until they either died or reached maturity.

In performing this experiment only wingless mothers with a long line of wingless ancestors were used.[2] Thus the effects that may have been produced by having a mixed parentage (some wingless and some winged) were avoided. Particular attention was given to having as uniform nutrition as possible, all individuals were placed on wheat shoots reared fresh for each experiment from the same seed bag and no individuals were started on any plant that had unfolded its leaves. In order to avoid, if possible, any effects on metabolism due to humidity, the soil in the breeding cell was kept moist, thus insuring, it was believed, a humidity above 37 per cent.; a degree of humidity which should give no change in the rate of metabolism (Headlee, 1914). During the whole of the experiment the aphids, and of course the wheat shoots on which they were placed, were kept in greatly

[2] In looking up my records of the parentage of individuals I find that the exact grandparentage of lots used in the 60° F. experiment is not recorded; however, they were from a fraternity that included but two-winged females and were certainly almost entirely if not entirely of wingless grandparentage.

subdued light. The specially constructed asbestos-lined "oven" was lighted only by a minute opening which faced the side of a room many feet from the sunlight and the single outside window. And finally, the same mixed sand and soil was used for growing all the wheat plants.

As a result of these experiments the following points in regard to temperature were determined:

(1) That the optimum temperature for the development of the wingless forms was near 65° F.

(2) That this temperature was shown to be a very important factor affecting the ratio of wingless to winged forms produced.

(3) That the percentage of winged individuals produced increased as the temperature varied both ways from the optimum for the production of wingless forms, which optimum was very near, if not the same as, the optimum for greatest metabolic activity (velocity of chemical reaction (?) Sanderson, 1910).

Further, as a result of these temperature experiments on individuals of a single strain, of a single race with a known parentage for 16 generations or more, and of a uniform ancestry of wingless parents, it was decided to test further the effects of temperature by its regulation in the small room in which the experiments were conducted so as to avoid as far as possible large variations from the optimum of 65° F. An endeavor was made to see if many winged individuals would be produced under these conditions. In this second experiment, which was in the nature of a compound one, for I was selecting for a definite morphological character in order to test the pure line theory of inheritance, no special precautions were taken in regard to the control of other factors than temperature. But, however, it is to be noted that each succeeding generation was started on tender shoots, as had been my practice for months before the temperature experiments were conducted.

As a result of this second experiment I was able to propagate the line for 20 generations without the ap-

pearance of a single winged individual. In the 16 generations obtained before the temperature experiment was started, winged forms had appeared in 6, and in the case of one generation constituted all the individuals, and in the case of another generation a majority of the individuals of the fraternity.

The results obtained in regard to the effects of temperature were presented in less than 2 pages in my 1916 paper. At the time the experiments were made considerable importance was attached to the results, but because the number of individuals used was small it appeared advisable not to give them undue importance in the printed form. Viewed in the light of the subsequent work by others, and particularly in the light of Wadley's experiments on the same species, the experiment has a much greater significance. I think that it should be particularly valued; as it is, apparently, the only experiment that has been performed on the effects of an environmental factor in influencing the production of apterous or alate asexual forms of plant lice, in which individuals were used that had the same parentage (in regard to the presence or absence of wings) for a long series of generations previous to the test of controlled factors.

Wadley's Results

Before considering further my experiments on parentage, temperature, etc., it is best here to review Wadley's results along the same line. Wadley (Wadley, 1923), carried out a series of experiments on the effect of temperature on the dimorphism of asexual females using the temperatures of 62° F, 65° F, 70° F, 72° F, 75° F and 80° F. In his experiments he used individuals of four kinds of parentage; those with both grandparent and parent apterous, those with grandparent alate but parent apterous, those with grandparent apterous but parent alate and those with both grandparent and parent alate.

In regard to his results: In general they parallel those of mine, as he states in his paper; however, there is this

very important difference. The percentages of alate individuals produced in his experiments is much lower than in mine. In explaining this difference he suggests that it might be attributed to a possible lack of nutrition control in my experiment. This, however, could hardly be the case, as I made a particular effort to see that the individuals had the same kind of food.

When I first observed his "parallel" comparison of our results (Wadley, 1923, p. 291), it was difficult for me to explain the differences, but in looking at his table on the bottom of page 287 (Wadley, 1923) it became apparent. The figures given for his experiments on page 291 (Wadley, 1923) are averages for all individuals regardless of parentage. My experiments were with individuals having a long line of apterous parents. (However, this fact was not clearly stated in the 1916 paper.) When we compare his results for individuals that had both parents and grandparents apterous with my results there is a much greater conformity.

In order to further analyze the effects of parentage I will give in this paper the results obtained by both Wadley and myself in the form of a plotted curve, but first I shall present the pedigree of the individuals used in my temperature experiments. This is here given (Table I) for the first time.

Only wingless forms were used as parents for all the individuals born and reared in the constant temperature experiments.

In the pedigree chart it is seen that in the case of three of the four sets of ten individuals used as parents in the constant temperature experiments the parentage of each ancestor for many generations back is known and each of these was a wingless individual.

In the case of one set of ten parent individuals, the set used for the 60° constant temperature experiment, the exact parentage of these mothers (those from whom the individuals at the constant temperature were born) is not known. However, the grandparents are known to

TABLE I

PEDIGREE OF APHIDS USED IN TEMPERATURE EXPERIMENT

Number of generation of isolation	Key number and status as to wings of ancestral parent	Date of selection of parent and the isolation of her young offspring	Number of offspring	Status of adult offspring. Wingless	Status of adult offspring. Winged	Died or lost in infancy
I	10_{39} 9 wingless	Aug. 4 (1914)	17	0	15	2
II	11_{40}10 winged	" 17	3	3	0	0
III	11_{41} 1 wingless	" 28	6	1	0	5
IV	11_{42} 1 "	Sept. 6	9	8	0	1
V	11_{43} 3 "	" 13	13	9	0	4
VI	11_{44}10 "	" 20	10	9	0	1
VII	11_{45}10 "	" 27	14	7	1	6
VIII	11_{46} 3 "	Oct. 4	12	5	4	3
IX	11_{47} 4 "	" 11	21	14	0	7
X	11_{48} 5 "	" 18	13	8	1	4
XI	11_{49}11 "	" 25	16	13	0	3
XII	11_{50} 1 "	Nov. 1	25	20	0	5
XIII	11_{51}12 "	" 8	28	22	0	6
XIV	11_{52}10 "	" 15	26	22	0	4
XV	11_{53} 8 "	" 22	11	9	2	0
XVI	11_{54} 3 "	" 29	28	11	15	2
XVII	11_{55}25[3] "	Dec. 6	16	3	10	3
XIX	11_{56} 8 "	" 13	19	10	6	3
XX	11_{57}11 "	" 20	18	18[4]	0	0
XXI	11_{58}11 "	" 31	5	5	0	0
XXII	11_{59} 3 "	Jan. 7 (1915)	21	16	0	7
XXIII	11_{60}15 "	" 13	15	14[5]	0	1
XXIV	11_{61} 6 "	" 20	11	10	0	1
XXV	11_{62} 3 "	" 27	13	13	0	0
XXVI	11_{63} 2 "	Feb. 3	18	15	0	3
XXVII	11_{64}15 "	" 8	8	8	0	0
XXVIII	11_{65} 1 "	" 13	14	13	0	1
XXIX	11_{66}12 "	" 19	15	15[6]	0	0

[3] Parents of 10 mothers used in 60° constant temperature experiment among fraternity of this individual.

[4] Ten of these 18 wingless individuals used as mothers for 70° temperature experiment.

[5] Ten of these 14 wingless individuals used as mothers for 80° temperature.

[6] Ten of these taken for 90° constant temperature experiment.

have been among the individuals of a fraternity of 11 individuals only two of which were winged. That some of these 10 mothers came from a winged form is possible but not probable. The chances are against it. But even if some of these did come from a winged parent the number could not be large enough to affect seriously the results of the experiment.

When we compare the results obtained in my temperature experiments with individuals of apterous monomorphic parentage with the parentage control temperature experiments of Wadley we get the following results:

Percentage of Progeny Alate at all Temperatures

From alate parents and grandparents (Wadley)	0 per cent.
From alate parents only (Wadley)	2.28 " "
From apterous parents only (Wadley)	6.07 " "
From apterous parents and grandparents (Wadley)	9.24 " "
From apterous parentage for 16 or more generations (Ewing)	30.38 " "

Thus it is noted that there is a summation effect of parentage on the offspring of a generation of aphids depending upon the number and nearness of the ancestors of either form. Two generations of alate ancestors gave 0 per cent. of alate offspring. Where the parent only was alate a percentage of 2.28 was obtained. The immediate parent being an apterous individual gave a percentage of winged offspring of 6.07; both parent and grandparent being apterous gave a percentage of winged progeny of 9.24; apterous parentage for 16 or more generations gave a percentage of 30.38 of alate offspring.

When these results are analyzed as to temperature effects and the whole plotted in the form of curves, the effects are more easily comprehended. These plotted results (Table II) show in an excellent manner not only the striking effects of temperature but the equally striking effects of parentage on the ratio of alate and apterous individuals produced.

Undoubtedly there were several factors, such as quality of food, nature of soil used to grow wheat plants and

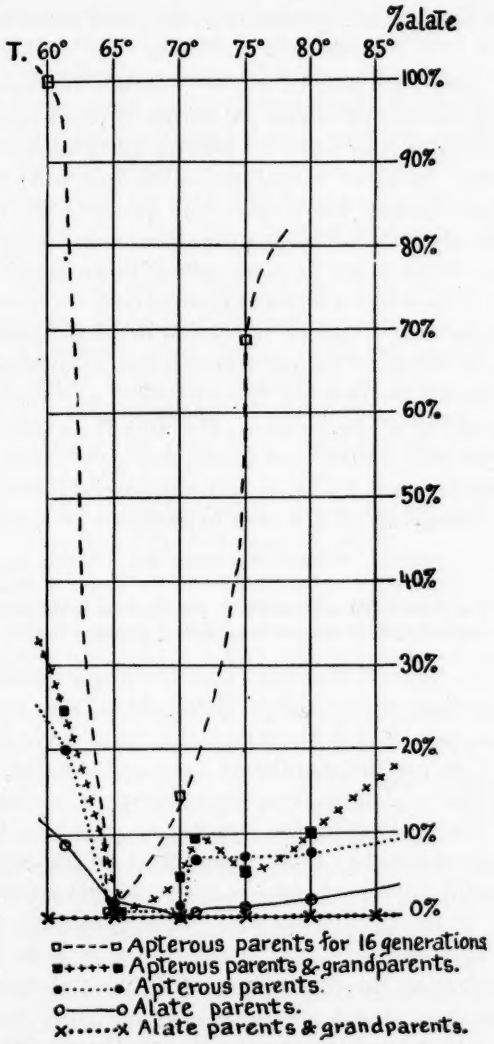

FIG. 1. Plot showing effects of temperature and parentage on the percentage of alate individuals produced (given in column to right) of apple-grain aphid. (Data from Wadley and from Ewing.)

light and evaporation that were quite different in these experiments, but their remarkable conformity in regard to both temperature and parentage is very striking.

THE EFFECT OF TEMPERATURE ON THE PRODUCTION OF SEXUAL FORMS

The apparent effect of colder weather in the autumn on the production of the sexual forms of plant lice in temperate climates was noted by several entomologists many years ago. In more recent times various workers have found that species which normally produce the sexes in the colder sections of the temperate zone reproduce without them from year to year, when these same species occur in tropical or subtropical countries.

Uichanco (1921) has accumulated much evidence in regard to the effect of temperature on the production of the sexes. He claims that but few aphids of any species produce the sexes in the tropics. He cites the experience of Goot, who after three years of biological work on the Aphididae in Java failed to find any sexual forms in that island. Speaking of his own experience he states that:

> Likewise, several years of casual observation and collecting in the Philippine Islands by me failed to disclose the male and oviparous female forms. So far as I am aware, the occurrence of amphigonous aphid individuals in any other tropical country has not been definitely reported.

While in general it is true that the majority of aphids do not produce sexual forms in the summer in temperate countries, yet in a few species the sexes have been reported even for the months of July and August (Patch, 1920, p. 162). These exceptions, however, could hardly be used for supporting the hypothesis advanced by some that temperature has little or no effect on the production of different forms of aphids. The observations made, not only in the tropics but also in the temperate regions, undoubtedly indicate that temperature is a very great factor affecting the production of different forms.

As has been stated previously in this paper the apple-grain aphid reproduces on the Pacific Coast without the appearance of the sexes. Relative to this statement it would appear pertinent to record the following observation made at Corvallis, Oregon. During the winter of 1913–14 large numbers of Coccinellidae were carried over

for various purposes to the following spring. In order to secure for these Coccinellids an ample food supply a screened-in, out-of-door bed of wheat plants was infested with the apple-grain aphid during 1913. Here the aphids multiplied by the thousands and passed the entire winter without a single sexual form or egg being found. The bed of wheat was several feet long and almost as wide. The wheat plants grew until finally they were almost a foot high.

Later (1914) at Ames, Iowa, the effect of temperature was tried. On December 11, the offspring of 11_{56} 7w, a winged individual, were taken with the potted wheat plants on which they were feeding and placed on a window-sill and the window closed. The nymphs grew slowly, but with the coming of temperatures near zero Fahr. were all killed.

Wadley (1923) attempted to produce the sexes in the apple-grain aphid but was unsuccessful. He states (p. 302):

> With the species used by the writer, cold, hunger or long-continued parthenogenesis did not produce the sexes and artificial colonization on the winter food-plant was ineffective. Just what combination of factors will produce the sexes is yet to be found.

Experimentally, Davidson (1924) has shown that lowering of the temperature may cause the appearance of sexes in *Aphis rumicis* L. After propagating a pure line of this species from November to January 15 under artificial light conditions and at higher temperatures, during which time only agamic individuals appeared, he found that after the placing of the individuals under conditions of lower temperature sexual forms appeared in the generations from February 10 to June 10. Davidson came to the conclusion that "it seems highly probable that sunshine, temperature and length of day are influential factors" affecting the appearance of the sexes and affecting the ratio of agamic to gamic individuals.

Effects of Nutrition

Early in his work with the apple-grain aphid the writer observed that those nymphs which settled upon the ex-

panded wheat leaves or those that fed upon withering plants were more frequently winged than those that fed upon the succulent shoots. Later the effect of this type of feeding appeared to be demonstrated incidentally in one of the heredity experiments.

In isolation 11, after five generations of individuals in which not a single winged form appeared, suddenly in the seventeenth generation 15 individuals out of 26 became winged. One of the wingless individuals of this fraternity was selected as a parent. From it even a larger percentage (10 out of 13) were winged.

In order to explain this sudden appearance of winged forms the conditions of their rearing were examined. First, temperature was suspected, but it was found that the average daily mean was 73.04° F. This was not very far from the optimum and should have given only a small percentage of individuals winged. Then it was noted that due to the lack of proper watering of the soil the wheat plants had been allowed to wither and shrivel. This was the only unusual condition that had been noted and was very probably the cause of the sudden appearance of these winged forms.

Taking advantage of this experience greater care was given the plants and not only were they kept well watered, but the most of the first formed leaves were clipped off to prevent the aphids from feeding on the harder tissue of the leaf surface. As a result of these precautions several generations were again procured without the appearance of a single winged form.

The effects noted in this experiment were later verified in a way in the starvation experiments of Miss Gregory (1917) and of Wadley (1923), the later authority using the same species as the writer. So impressed was Miss Gregory with the effects of starvation that she assigned to nutrition the major rôle in the altering of the ratio of form in aphids.

In regard to the relative influence of temperature and nutrition on the production of winged agamic aphids

there has been a wide divergence of opinion. Miss Gregory states:

> In conclusion it seems most probable that the lessening of the food supply is the primary factor in determining the development of wings in the offspring of wingless mothers. The wing anlage appears to be present in all the parthenogenetic females and depends directly upon the condition of the food supply of the mother for its stimulation or suppression of development.

On the other hand, some would hold that nutrition is of much less or even minor importance. Thus Baker and Turner (1916), working with *Aphis pomi* De Geer, found that lack of proper nutrition did not cause the winged forms to appear. They state (p. 976):

> The theory has been frequently advanced that the production of winged forms during the summer is due to a lack of sufficient nourishment for the insects. In some cases the wording of this theory is modified by the statement that winged forms appear on plants which are very heavily infested. The writers' results are a flat contradiction of this theory for this species. As has been stated previously, in handling the insects the writers always transferred the mothers to new plants, rather than the progeny. In this way several consecutive generations were reared on one plant. Thus the effect of poor or good food would be accentuated. Yet the winged forms were never obtained in series of small, poorly fed insects, but occurred frequently in well-nourished series.

Wadley, working with the apple-grain aphid, found, (page 297) that:

> A high percentage of winged forms was produced by starvation at all temperatures tested, but greater difficulty was encountered in producing alate aphids at 65° than at other temperatures.

Summary in regard to Factors affecting Production of Forms of Plant Lice

Up to the present we have but little experimental evidence in regard to the factors which influence the appearance of sexual forms of plant lice. This evidence, such as it is, indicates that the factors affecting the appearance or percentage of sexual forms are similar to those affecting the ratio of the different forms of agamic individuals.

In regard to the factors affecting the ratio of winged to wingless agamic females much experimental evidence

has been produced showing that there are several of these and that definite results can be obtained in the laboratory by modifying any one of them.

The percentage of either one of the agamic forms produced in any fraternity depends first of all to a very large degree upon parentage and ancestry. Temperature is a very important factor, in certain species, at least. For the production of wingless forms there is an optimum temperature, and a variation from this optimum in either direction causes an increasing number of winged forms to appear. At the optimum temperature for the production of wingless individuals there is a tendency of succeeding generations to approach 100 per cent. as a limit for this agamic form, and at such a temperature other factors lose most of their effect. The limitation of the food supply is of much importance in the production of winged forms, particularly at temperatures far from the optimum. The nature of the food from the standpoint of chemical composition affects the ratio of the two types of agamic individuals. Indirectly, probably, light and evaporation are of considerable importance as form-producing factors. Both of these, however, are very intimately associated with temperature. Light alone may affect either the aphids directly or indirectly through its effect on the growing plants on which the aphids feed.

Among the outstanding factors that have been demonstrated experimentally as affecting the ratio between the two agamic forms of aphids are inheritance, temperature and nutrition, and the relative importance of each of these appears to depend on the conditions imposed by the combination of all other factors. Either inheritance, temperature or nutrition may under certain conditions become the dominant or even the controlling factor.

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HEREDITARY ANEMIA IN MICE AND ITS RELATION TO DOMINANT SPOTTING

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CUÉNOT, in 1905 (2), first pointed out the absence of homozygous individuals among yellow mice, and subsequent investigations have shown the existence of a similar lethal factor in mice of the dominant white variety. Many attempts have been made to account for this deficiency, but it was not until 1910 that Castle and Little (1) attributed the lack of homozygous yellow mice to the lethal action of Y genes in the duplex condition. They presented convincing evidence that "the yellow egg which by chance has met a yellow sperm has its career ended thereby." Studies by Kirkham (5) have showed that some ova die after implantation, and these he identified as the missing homozygous yellow individuals. His work was later confirmed and extended by Ibsen and Steigleder (4). All these investigators agree that homozygous yellow mice are produced but die during early embryonic life, the exact cause of death remaining wholly undetermined.

In 1915 Little (7) revealed the presence of a lethal factor in the dominant white strain of mice. This factor associated with dominant white spotting he found to be genetically distinct from the lethal factor associated with yellow coat color. In 1919 (9) he presented evidence to show that while genetically distinct the two lethals produced the same phenotypical effects, the young in both cases dying soon after implantation.

In the course of breeding experiments with dominant white mice in this laboratory the appearance of aberrant offspring was noted. The work embodied in this paper was undertaken at Professor Danforth's suggestion as

a first step towards an understanding of their genetics and embryology.

Since this study was begun, Detlefsen (3) reported that "in strains of black-eyed [dominant] white mice a type of young has occurred rather frequently, which invariably dies one to three days after birth. Their appearance is distinctive and they can always be identified, since they are about one half normal size and present a very white and bloodless appearance. Their occurrence suggests that the homozygous black-eyed white (which is lethal as in the case of the homozygous yellow) may perhaps develop in some instances far beyond the stages supposedly characteristic of the homozygous yellow."

The main part of our stock is homozygous for recessive spotting, which occurs in black and brown, modified in about half of the cases by the "pink-eyed dilution factor." The factors which have a bearing on the data reported in this paper are:

D, dominant spotting; d, the allelomorph for dominant spotting.

P, dominant black eyes; p, the allelomorph which produces pink eyes.

DdPP and DdPp individuals are usually pure white with black eyes, but occasionally show some dark hairs on the rump and about the ears.

Ddpp mice are nearly always pure white but may have some light-colored hairs on the rump and about the ears. The pure white individuals resemble albinos, but microscopic examination of their retinae seems to show traces of pigment, and when mated together they produce some spotted young.

ddPp specimens have black eyes with spotted pelage; those with the formula ddpp have pink eyes with spotted pelage. The exact shade in the last two cases depends upon the presence or absence of P. The color of the eyes can be determined in early fetal stages.

My experience has been in accord with that of Little (7) and other students of dominant spotting in that I

have been unable to obtain adult DD mice, but I noticed from the first that from Dd × Dd matings there were a certain number of aberrant offspring, a result corresponding with Detlefsen's findings.

In a total of 75 aberrant young born and observed in this laboratory, it has been found that these abnormal individuals consistently differ from the normal both in appearance and in length of life. Their skin is paler and contrasts strikingly with the deep pink color of the normal new born. When the blood of the abnormal young is compared with that of the normal from the same litter by means of a von Fleischl hemoglobinometer the readings are about 22.66 and 103, respectively. Because of the difficulty of obtaining sufficient blood for standard readings from an anemic individual, corresponding amounts of normal blood were used as a standard. Since the technique is identical, the figures form a reliable basis for comparison. Readings obtained with the Tallquist scale were essentially the same. Lange (6) in his work on the hemoglobin content of adult mouse blood obtained figures closely approximating these findings for the normal. The erythroplastids in the anemic blood averaged from 20 to 25 per cent. of the number found in blood from normal young in the same litter.

Intermediate phases were not present in any of the 75 cases observed, the true anemia being distinctive in appearance and therefore readily identified. That this anemia, although hereditary, is not comparable to the hereditary sickle-cell anemia of man described by Taliaferro and Huck (10) is indicated by the fact that the red blood corpuscles in the blood of the anemic mice do not assume elongated or crescentic forms in hanging drop preparations. Anatomically, the anemic differ from the normal young in having a smaller thymus and a larger heart. The spleens apparently show no hypertrophy, but the study of these and other organs is still under investigation.

The number of anemic individuals which appear in a litter is not constant, four out of seven being the highest

number observed. No correlation was found between anemia and eye color or sex. The span of life usually varies from a few seconds to five days, although in one case an anemic mouse lived to be eight days old. At the time of birth they may be fully the size and weight of the normal young, but more frequently they are smaller. Despite the fact that they often ingest relatively large amounts of milk, each succeeding day finds them thinner and more emaciated until finally they die. We have not been able to prolong their life even by removing all normal young from the litter and leaving the adult female with only one or two anemics to nurse. The relative size of the normal and anemic young on successive days is shown in the figures. The litter of six, from which two normal mice were removed at birth, was from a Dd × Dd mating. The first photograph was taken two hours after birth, and the subsequent pictures at approximately 24-hour intervals.

The percentage of anemic individuals from different matings is indicated in Table I. The total percentage of

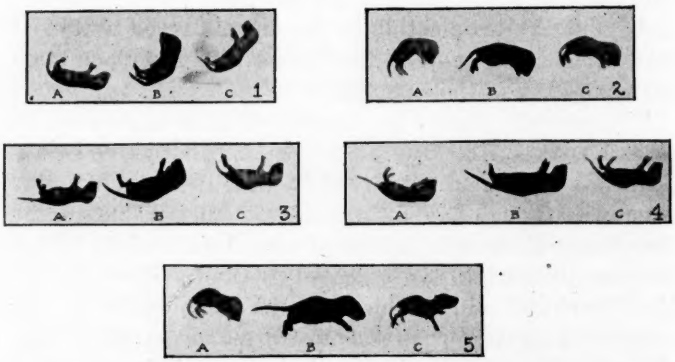

FIG. 1. Photographs of one normal and two anemic young from a Dd × Dd mating. All the pictures are made to the same scale; the first was taken two hours after birth, the following ones at successive 24-hour intervals. The three individuals are in the same relative position throughout, the normal control being in the middle. Both of the anemic young died before the end of the fifth day without increase in size. The usual amount of growth during this period is shown by the control.

TABLE I

OFFSPRING FROM Dd × Dd AND dd × Dd MATINGS

Mating	Number of matings	Total young	Normal young	Anemic young	Percentage anemic	Average size of litter
Dd × Dd	122	500	425	75	15	4.098
dd × Dd	38	185	185	0	0	4.86

anemics was 15, but the frequency varied during the period of observation, being higher (17 per cent.) when the cages were inspected two or three times a day and lower (10 per cent.) when observations were taken once every 24 hours. This apparent variation was probably due to a differential death rate at or immediately following birth. Even the most frequent observations are not wholly accurate, for there is always the possibility of some young being eaten or lost before they can be observed. The average size of litters in both types of matings is shown in Table I. If the prenatal death rate is the same for both anemic and normal individuals, the expected size of the litters for the two groups should be identical, which condition closely approximates what was actually found. The insignificant discrepancy observed probably is due to the fact that some of the anemics were lost after birth.

To overcome the difficulties encountered in trying to tabulate accurately the young born, an attempt was made to determine whether anemics could be distinguished from normal fetuses in utero. The total gestation period of these mice is about 20 days, and by recording the time of mating the state of pregnancy can be ascertained. The pregnant females may then be opened and examined under an anesthetic and the condition of the fetuses observed. It was found that anemics are readily distinguishable from normals by the sixteenth day and that the living fetuses can be recognized as such both by their appearance and their movements. All the figures used in Table II are based on fetuses 16 days old or older.

Besides fetuses from parents both heterozygous for D, fetuses from dd × Dd matings were also examined. The results recorded for each group are indicated in Table II.

TABLE II

FETUSES FROM Dd × Dd AND dd × Dd MATINGS

Mating	Total fetuses	Total living fetuses	Normal living fetuses	Anemic living fetuses	Dead implantations	Percentage of anemic among living fetuses	Percentage of dead implantations
Dd × Dd	270	211	159	52	59	24.64	21.85
dd × Dd	102	79	79	0	23	0.00	22.54

These figures show that in litters where there was the possibility of homozygous D genes anemics were present, but that in litters resulting from mating the same males to dd females, anemics failed to appear. The conclusion which this observation justifies is that the anemic young are those which are homozygous for D and that this anemia, which is hereditary, is due to homozygosis of the D gene or one closely linked with it.

Theoretically, the proportion of homozygous D's is 25 per cent., a proportion which corresponds almost precisely to the number of anemics found (24.64 ± 1.96). This close correspondence indicates that such fetal deaths as occur before the sixteenth day are uniformly distributed between anemics and normals and that the lethal effect of homozygous dominant spotting does not appear until the end of pregnancy. This is quite different from what has been previously supposed.

That the anemia is wholly a matter of heredity and not in any way dependent on defective diet seems to be clearly demonstrable. All the stock used was under one year of age. A certain number of both the Dd and dd groups were fed very carefully from the time of weaning, their food including milk, butter, cheese, lettuce, raw meat, sunflower seeds, peas, corn, wheat and mineral

salts, but no difference was noted in either the number or the appearance of anemic young among litters of the Dd females when mated to Dd males. In many cases these Dd and dd females mated with the same males. The Dd females constantly produced some anemics, while the dd females produced only normals.

The anemic factor was also transferred to a strain in which both parents, brother and sister, had descended from a wild female and a Dd male. From the F_1 generation, in 13 uteri, 73 normal and 6 anemic fetuses were obtained. Among four litters born alive 29 normals and 6 anemics were found. The F_1 generation, back-crossed to dominant white mice, produced eight normals and one anemic.

Besides the normal and anemic fetuses in the uteri of dominant white mice, there are found embryos which have died after implantation. From Table II it will be seen that in Dd $\times$ Dd and dd $\times$ Dd matings the dead embryos represent, respectively, 21.8 and 22.5 per cent. of the total. Since the percentage of anemics found in utero is 24.6, a higher prenatal death-rate among this class could hardly account for the number of dead implantations, and since they occur in about equal frequency in both types of mating the possibility of their being associated with the homozygous lethal factor seems to be excluded.

Overcrowding has been suggested as an explanation for this form of prenatal mortality, but if this were the case one should find the number of dead fetuses far greater among the larger than among the smaller litters. From 112 pregnant uteri examined 50 contained litters of seven or over. Of these 50, 60 per cent. contained dead implantations, while from the remaining 62 uteri, including litters of six or less, 51 per cent. contained dead ova. Hence overcrowding does not seem to have been a very significant factor.

Similar fetuses which died early in gestation were reported by Ibsen and Steigleder (4). Some of these they identified as the missing homozygous yellows. There remains the possibility that many of the dead implantations in the uteri of dominant white and other mice represent a third lethal, the somatic appearance of which has not as yet been recognized. This is rendered the more probable, since in the inbred Dd and dd strains the incidence of this class approximates 25 per cent.

SUMMARY

The homozygous young of the dominant white mouse ordinarily live throughout the whole period of gestation and not infrequently for several days after birth. No evidence is found that their prenatal death-rate is greater than that of the normal individuals of the same litter. They are characterized, at least from the sixteenth day of gestation, by a special form of anemia which is the real cause of death.

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ACCRETION AND DISTENTION IN PLANT CELLS[1]

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GENERAL FEATURES OF GROWTH

THE fact that two distinct phases or stages, accretion and distention, may be recognized in the growth and development of the plant cell has been discussed in papers published during the last two years.

In the earlier accretion stage material is added to the protoplasmic mass, and changes in volume are due to imbibition. The young cell quickly passes into the distention stage, in which its volume is increased many times by the osmotic action of substances in solution in spaces, cavities or vacuoles in the protoplasm. The rate of increase reaches its maximum in this second stage; the distending pressures set up as a result of the relative permeability of the plasmatic layers and wall may be as much as 70 or 80 atmospheres, and great rigidity in such cell-masses is produced.

The actual amount of material or dry weight of the protoplasm shows no increase after distention or ballooning begins. On the contrary, it begins to shrink. This wasting of living material proceeds at the slowest rate in cells which show the least differentiation and remain in a parenchymatous condition. In highly differentiated tissues which serve mechanical purposes chiefly, the wasting or consumption of the protoplasm continues, so that wood-cells or tracheids, vessels, etc., soon reach a stage where only a trace of the reversible gels which make up protoplasm remain.

Measurements of growth which cover more than one cell would include the resultant rates of units in overlapping stages of accretion and distention.

[1] Read by invitation before the American Society of Naturalists, Washington, D. C., January 1, 1925.

Increase in proportionate dry weight, as in animals, generally accompanies the development of the individual as in plants. The exceptions to this procedure are much more important among plants than among animals, and in many fruits, stems of succulents, sporophytes of the fleshy fungi and filamentous organisms the proportionate amount of water may be greatest at maturity.

The Initial of Fundamental Process upon which Growth rests

An attempt will be made on the present occasion to characterize somewhat more fully the physico-chemical processes which cause or characterize the two phases of growth of the cell. In so doing it seems hardly necessary for me to disclaim any idea that I may be presenting a completed scheme of metabolism, or a description of a continuous chain of events. I can scarcely do more than say that in the accretion stage, first protein and later carbohydrates are formed by condensation and synthesis and dehydration, and that in the second these substances are hydrolyzed and expanded. Some of these features have come in for discussion by several writers, but it may be profitable to make the picture as complete as possible at this time.

The union of molecules of two amino-acids in the formation of a protein would result in the formation of larger particles with a capacity for holding water less than the total of the separate molecules with a consequent dehydration. This would entail an accompanying or consequent setting free of water in the condensation or accretion stage of the protoplast. In such continuously acting cell-masses as the cambium the freed water would be taken up in the hydration of the older daughter cells which would be in a stage of expansion or distention by the accumulation of water in the syneretic spaces or vacuoles. Such a flow would in the case of the cambium be from protoplasts with a lower H ion concentration to a higher one.[2]

[2] Pearsall, W. H., and Priestley, J. H. "Meristematic tissues and protein isoelectric points." *The New Phytologist*, 22, 185. 1923.

The interlocking processes of accretion and distention are not, however, simply condensations and hydrolyses of proteins.

The activity of plant cells is notably characterized by the accumulation of carbohydrates as a constituent of the cell mechanism in greater proportion than in animals. It is true that combined carbohydrates occur in all nuclei, and that mucilages occur in the development of eggs, from the study of which the zoologist derives most of his cytological conclusions, but the condensation of sugars in the deposition of cellulose material in cell-plates and in wall-formation, as well as in the accumulation of the pentosans, mannosans, glucosans, pectins, etc., in the mass, is a procedure most noticeable in plant cells.

The hydrolyses of the proteins of the cytoplasm has been attributed to the action of basic proteins emanating from the nucleus by Spek. The hydrolysis of the cytoplasm in eggs is shown by Wilson[3] to follow the dispersion of the chondriosomes from their clustered position about the nucleus, who says:

> One might imagine, for instance, that during this association (*chondriosomes with nucleus*) the formed elements receive from the nucleus certain substances (enzymes or other chemical messengers) of which they become the carriers transporting them to regions of the cytosome where they play their specific part.

These speculative conclusions and disconnected observations are harmonized by my own observations that biocolloids consisting of proteins and pentosans show a coefficient of hydration in the presence of such amino-compounds as glycocoll, alanin, phenyl-alanin and histidin far in excess of that in water alone.[4] These compounds adsorbed by the chondriosomes would be carried toward the periphery of the cell until freed by meeting penetrating electrolytes.

The intrusion of the electrolytes also establishes new conditions of permeability in the protoplast. The plas-

[3] Wilson, E. B. "The Cell," 2nd ed., p. 346. 1925.

[4] MacDougal, D. T. "The distentive agencies in the growth of the cell." Proc. Soc. Exper. Biol. and Med., 19, 103. 1921.

matic layer in which the proteins, pentosans and lipins are intermixed takes on a condition as a result of the interlocking and to some extent interfering action of the ions of K, Na, Mg, Ca and other bases as well as acid radicles in which it is variously permeable to these materials and all but impermeable to organic substances.

Obviously a distending cell will reach its greatest turgidity when the complex plasmatic layer is most nearly impermeable to the dissolved substances in the cell, the activities of which result in the attraction of water. Such a condition of permeability has been thought by some writers to be coincident with an isoelectric condition of the proteins alone.

It seems to me so obvious that the permeability of a layer must depend upon the degree of hydration of its constituents that it does not appear profitable to elaborate the statement. Furthermore, differences in potential or the electric charges on the particles may originate in so many ways that I am not able to share the assumption that the activity of the hydrogen ion is the sole determining factor in the realm of physiological action. Its speed and penetrating action is greatest but in any mass of protoplasm its action may be controlled or masked by that of other carriers or electrolytes.

Isoelectric Zones

The gradients of hydrogen-ion concentration in the growing regions of stems are of the greatest interest. Thus the phellogen or embryonal layer which produces cork shows a high acidity denoted by Ph 3, equivalent to 0.001N HCl. Internally to it the cortex has an index of Ph 5.5 to 6.5. With regard to the vascular cambium, the phloem formed by it may be alkaline, Ph 7.8, and the xylem or woody cells as acid as Ph 5 or even Ph 4.3.[5] Among the more important implications are those which are concerned with the isoelectric points of the proteins. These substances would be condensed in the cells in which

[5] See Pearsall and Priestley, as cited above.

the hydrogen-ion concentration is identical with their isoelectric points, in which stage cells of protein alone would be least permeable. This is directly contrary to the statement by D. Haynes that permeability is at its maximum in a colloid in an isoelectric condition.[6]

Robbins has taken the maximum turgidities in cells to lie in the region of the isoelectric zones of proteins properly implying the condition of least hydration but in disregard of the effect of the presence of other material affected both by the hydrogen-ion concentration and the presence of electrolytes. A further series of observations of value in this connection was made by O. Arrhenius,[7] who found that the relation between the growth and Ph gave in most cases a curve with two maxima, so that a majority of plants seems to have two optimum hydrogen-ion concentrations—*e.g., Pisum sativum* at Ph 6 and Ph 8, *Avena sativa* at Ph 5 and Ph 8, *Phleum pratense* at Ph 4 and Ph 9. Only one optimum is shown by *Lupinus luteus* (at Ph 6) and *Trifolium pratense* (at Ph 6).

The behavior of a protein cell is, however, a purely theoretical condition. The protoplast of the higher plant is a complex colloid, including a number of substances in the condition of a reversible gel.

In support of the contention that these points of maximum turgidity or greatest growth do not necessarily lie within the isoelectric zones of the proteins, I may cite results obtained by placing living cell-masses of Opuntia in various solutions and by tests of various kinds with constructed cells.

The succulent flattened stems of Opuntia are at all times in a pronounced acid condition. The total acidity of the sap may vary daily from an equivalent of 0.67 cc KOH 0.1 N to 1.48 cc per gram of dry weight. In the

[6] Haynes, D. "The interpretation of electrical stimulation in terms of changes of hydrogen-ion concentration and the production of permeability in the plasma membrane." *Science Progress*, No. 70, pp. 223–233. 1923.

[7] D. M. in *Physiological Abstracts*, Vol. IX, No. 2, May, 1924. Arrhenius, O. Nagra bidrag til kannedomen om sambandet mellan markreaktionen och vissa kulturvaxters utoeckling. (*Meddelande No. 245 fran Centralanstalten pa jordbruksomradet*, Stockholm, 1923. 1–13.)

more acid condition distention is greatest in solutions at Ph 2.5: in the less acid condition the greatest distention is in solutions at Ph 3. On the other side of neutrality the greatest distention was in NaOH 0.0025 N — Ph 12, with no distinguishable difference which could be connected with the varying acidity. This is of especial interest in connection with the fact that a buffer situation exists by which the sap is maintained at about Ph 4. The higher maxima lies on the OH side of neutrality, an effect which might be due to the presence of Na and the formation of sodium proteinates.

Such cell-masses placed in solutions of neutral NaCl showed the greatest distention in concentrations of 0.005M in the more highly acid condition; in the less acid condition the zone broadened so that no difference could be established in the expansion of the cell-masses in concentrations ranging from 0.0025 to 0.01M. Addition of such salts to the acid solution was followed by a maximum distention at PH 3 throughout the range of the material.

The addition of neutral salts to the hydroxide solution was followed by the greatest distention at PH 8 in the more acid condition and PH 11 in the less acid condition.

When these data are compared with the action of the constructed cells, which I have described in detail elsewhere, some interesting parallelisms are found.[8]

When only pentosans are included in the construction of experimental cells endosmotic action which would have produced distention in a protoplast was greatest in solutions at Ph 3, and in Na OH solutions at Ph 11. The inclusion of gelatine in such cells was followed by maximum effects at Ph 2.5 in acid solutions and Ph 11 to 12. The addition of lecithin to the materials of the cell was followed by broad zones of maximum action lying between Ph 2.5 to Ph 3 and in sodium hydroxide at Ph 12 (0.0025N).

[8] MacDougal, D. T. "The arrangement and action of material in the plasmatic layers and cell walls of plants." Proc. Amer. Phil. Soc., 63, 76. 1924.

If now we take into consideration the action of dead cell-masses which are nearly comparable to the constructed cells, the maximum action increased in acid solutions above Ph 2 and in hydroxides beyond Ph 11–12.

Such conditions of low permeability and resultant high distention and turgidity of cells have been interpreted by Robbins[9] as being determined by the isoelectric zones of the proteins. As such distention, however, depends upon the permeability of the plasmatic layer as a whole, and as this layer includes not only proteins, but pentosans and lipoids as well as intermediates, I have taken the ground that the maxima of distention in plant cells occurs under conditions in which all the substances in the complex plasmatic layer show a minimum permeability. For example, one of the two isoelectric zones of gelatine lies about Ph 4.7 and a cell constructed of this material should show greatest distention in an acid solution near this concentration. The constructed cell, including gelatine in its "plasmatic" layer, shows a maximum distention in acid at Ph 2.5 to Ph 3. Gelatine has a second isoelectric zone at about Ph 7.7. But the constructed cell including it has its maximum in the alkaline condition at Ph 11. These results are generally parallel to those obtained in dead cell-masses.

The parallelisms in the action of living cells, of dead cells and of constructed cells are conclusive evidence as to arrangement and general condition of material in the plasmatic layers and wall of the cell. The constructed cells display a pattern of action midway between that of living and dead cells, with respect to the H and OH and salt concentrations. Some of these differences may be ascribed to the buffer situation in the living cell, which is altered in dead cells, and has not been imitated in constructed cells. The complex set of ions of the common metals, which variously interfere and interlock, likewise can not be easily duplicated experimentally.

[9] Robbins, W. J. "An isoelectric point for plant tissues and its significance." *Amer. Journal of Bot.*, 10, 412. 1923.

Periodicity

It is obvious that the period of development of an individual is a cycle, which in minute forms may be rounded out within an hour and in the great trees may stretch out over thirty or forty centuries. The periodicities or intermittent activities which are displayed by plants in regions with well-marked seasons are to be ascribed to alterations in the environment, or to variations in the food supply. Bloom and fruiting await the accumulation of material and seasonal variations in temperature, and are to be connected with no inherent property of protoplasm. Klebs has succeeded in flattening the seasonal curves of activity of some of our deciduous trees by cultivation under equable or uniform climatic conditions in greenhouses. It is commonly assumed that trees in tropical rain-forests show practically continuous growth, although this matter has never been subjected to critical examination. I have, however, made some records of the Monterey pine in the equable climate of the Coastal Laboratory at Carmel, California, of some importance in this connection. The behavior of one young tree in the habitat of the species may be cited in illustration preferably to a summarization of the activities of the large number under observation. These trees make new leaves on each year's extensions of the shoots which are connected with the wood of the previous year, and which persist, two, three or even four years. The tree is therefore evergreen and has normally at all times an adequate transpiratory and photosynthetic mechanism. The air temperature rarely drops to 0° C. and the cambium layer of the tree is usually at a temperature of about 9° to 11° C., at which growth proceeds at a high rate. The tree in question (No. 20) has a record growth from October, 1922, to January, 1925, in which there are no seasonal nodes. During a few hot dry days each summer the loss of water from the trunk masks the increases in the cambial region, but does not stop elongation. Winter storms late in January or in February are accompanied by low

temperatures which stop enlargement of the shoots and trunk but not of the roots. The warm day following the coldest will be characterized by a rapid enlargement, and the tree will emerge from a drought shrinkage within five or six hours. Briefly, this pine may be characterized as one in which there is no inherent periodicity in the activities of the cork or vascular cambium.

The main conceptions presented in this paper are as follows:

SUMMARY

(1) Growth, or enlargement and differentiation, consists essentially of two groups of reactions, synthesis or condensations of proteins and carbohydrates in the accumulation of material in initial elements preceding and following mitosis, and subsequent hydrolysis and distention.

(2) The formation of proteins and of lipins characterizes the earlier stage. Beginning with the appearance of pentosans in the cell plate, condensation of the sugars progresses with a consequent increase of mucilages in the plasma and cellulose of the wall. Hydrolysis or hydration of the pentosan-protein plasma may be tentatively attributed to the action of acidic or basic amino-compounds which, emanating from the nucleus, may be carried into the cytoplasm by the chondriosomes. These substances would be freed from the chondriosomes by incoming electrolytes and are known to cause a greatly exaggerated swelling of biocolloidal mixtures simulating cytoplasm.

(3) Permeability, which is greatest in the youngest or undifferentiated stage, lessens toward maturity, probably by the action of absorbed and adsorbed electrolytes.

(4) The greatest distention of a cell takes place not precisely in the isoelectric zones of its proteins, but at a hydrogen-ion concentration and under other conditions of electrolytic action in which the complex layer is in a condition of least permeability.

(5) The actual dry weight of a protoplast does not increase during the distention stage which may be identified with increasing hydrolysis of proteins and condensations of carbohydrates, some of which go into wall formation. In elements of the xylem or wood the plasmatic material is entirely converted into wall structure. In some instances hydrolysis operates to give cells, organs or entire plants a proportionate higher water content at maturity than in the earlier stages. The nature of the catalyst which causes the hydrolysis of proteins following cell-division is not known. Similar hydrolyses are caused by basic and acidic proteins amino-acids and very dilute hydroxides, acids and neutral salts.

(6) The earlier changes in volume of the protoplast are due to imbibition and swelling. The later increases are the result of the osmotic action of substances in solution in syneretic cavities or vacuoles. Both are due ultimately to differences in forces referable to vapor pressure.

(7) Turgidity or increase in volume of the cell is determined by the permeability of the plasmatic layers and walls, especially to the substances in the cell-sap, and is the resultant of the combined action not only of the hydrogen-ion but of all charged particles or ions which enter into or impinge on the cell on the one hand, and by the activity of the cell-sap on the other.

(8) Records of growth in trees show no variations indicative of an inherent periodicity in living matter.

WHY POLYPLOIDY IS RARER IN ANIMALS THAN IN PLANTS[1]

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A CONSIDERABLE number of cases of tetraploidy and also higher forms of polyploidy have been found amongst plants, some having been observed to arise in cultures that were under observation, others being found already established as varieties or species having twice (thrice, etc.) the amount and number of chromosomes present in related types. Evidence also exists that forms of plants thus established may give rise to larger subdivisions (genera, etc.) inheriting all this chromatin, or even more, since the doubling process may be repeated. On the other hand, amongst animals, cases are very rare where there is critical evidence for the occurrence of this evolutionary process.[2] Apparently this is not because doubling of chromosome number does not occur in cell division—for cases of tetraploid cells in diploid animals have been observed not infrequently. Neither is it likely that polyploid individuals would fail to live. For chromosome conditions in the Hymenoptera show that at least two chromosome numbers are equally viable there—haploid and diploid—and polyploid Drosophilae have been found by Bridges to be very vigorous.

What, then, is the reason for the rare occurrence of polyploidy in animals, as compared with plants? It is, in essence, very simple—animals usually have two sexes which are differentiated by means of a process involving the diploid mechanism of segregation and combination,

[1] Department of Zoology Contribution, no. 192.

[2] This contrast between animals and plants has also been pointed out by Gates in an interesting paper which has just come to hand: Gates, R. R., 1924, "Polyploidy," *Brit. Jour. Exp. Biol.*, 1, p. 153–182.

whereas plants—at least the higher plants—are usually hermaphroditic. Less simple are the details of the working out of this principle, for the diploid sex-determining mechanism and bisexuality act in various ways to hinder the establishment of tetraploid races, as will be shown below.

(1) The triploid individual is a usual intermediate step between diploid and tetraploid individuals in hermaphroditic organisms, the tetraploid in most cases arising by the self-fertilization of a triploid or by the crossing of two triploids. But this will often be impossible in bisexual organisms, for individuals having proportions of the sex-producing genes normal to the heterozygous sex can not exist in triploids and hence triploids of one sex or the other may fail to be fertile. This is due to the fact that the development of sex, as well as of other characters, depends upon a certain proportion existing between the materials formed by different genes concerned with that character—thus maleness is produced in XX-XY species when that proportion of the genes affecting sex is present which exists in individuals having one X and a pair of all the autosomes, femaleness when 2 X's and a pair of all the autosomes are present. This "ratio" view of the determination of sex in "XX-XY" and "WZ-ZZ" forms, which conflicts sharply with the hitherto prevalent notion of determination through absolute quantities of the sex chromosomes, had been urged by the present writer, in discussions with Drosophila workers, since 1912; experimental confirmation of it was finally obtained in 1921, in Bridges'[3] findings of triploid Drosophila. In these it is evident that neither the one X- nor the two X-containing triploids are normal sexually, though they have the absolute quantity of X-chromosome material that differentiates males from females among diploids; both these triploid forms are sterile whereas the 3X triploid is a fertile, vigorous female. Thus whenever a

[3] Bridges, C. B., 1921, "Triploid intersexes in *Drosophila melanogaster*," *Science*, 54, p. 252–254.

fertile triploid is formed in Drosophila it must be a female, and it can have no triploid males with which to breed. Breeding with a diploid male it produces only diploids, triploids and inviable or infertile diploid-triploid mixtures. Thus the numbers of triploids will tend to decrease in each succeeding generation, and no tetraploids will be produced. *Mutatis mutandis,* in WZ-ZZ species, triploid males may be produced, but these will lack fertile triploid females with which to breed, and the occurrence of tetraploidy will be similarly interfered with.

(2) It will occasionally happen, however, that a tetraploid animal will arise without the interbreeding or selfing of triploids. There are three chief methods whereby such an event may occur: (a) A diploid gamete from a fertile triploid parent may happen to meet an anomalous diploid gamete from a diploid parent in which, owing to a mitotic irregularity, doubling of chromosomes in the germ tract had occurred; (b) a diploid egg formed by mitotic irregularity in a diploid female may happen to be fertilized by a diploid sperm formed by an independent mitotic irregularity in a diploid male (such formation of diploid gametes is more apt to occur in species-hybrids than in "pure" types, and this is especially true in the Lepidoptera, where aberrant maturation divisions, resulting in diploid gametes, are not uncommon in hybrids); (c) a diploid fertilized egg may become tetraploid through mitotic irregularity occurring in early cleavage. The resulting tetraploid should sometimes happen to have 2X (or Z) chromosomes; in that case it will be of the type of the heterozygous sex. It may instead happen to have 4X's (or Z's), in which case it will be of the type of the homozygous sex.

In any of the above cases, once such a tetraploid has been produced, it will usually have to breed with diploids. Triploids will then result and continued breeding will lead into the genetic *cul de* sac mentioned in section (1). If, however, by a rare coincidence, two or more tetra-

ploids of different sex have been formed and they breed together, or if a tetraploid of the heterozygous sex type breeds with a triploid, tetraploids will be reproduced, and a race of tetraploids could theoretically be established from these. (This would occur more readily in the case of Lepidoptera, after hybridization, for the reason explained above.) Once formed, however, the race of tetraploids could only persist if their genetic isolation were continued in each generation, for interbreeding with diploids would immediately destroy the tetraploid combination, leading to triploids that would usually (again excepting cases like the Lepidopteran species-hybrids) tend to revert to diploids.

(3) If, however, all the above conditions for the establishment of tetraploid lines had been fulfilled the race of tetraploids would still be at a reproductive disadvantage, as compared with the diploids, and so would tend towards extinction under the conditions of a natural struggle for existence. The handicap of the tetraploid forms again lies in their sex-determining mechanism. For, in the tetraploids of the heterozygous sex type, containing, let us say, 2 X's and 2 Y's, there is nothing to insure all the gametes getting either both X's or neither.

As the writer pointed out in 1914, in discussing the mechanism of segregation in tetraploids,[4] four homologous chromosomes of 2 kinds, designated as A, A, a and a, should ordinarily segregate into two pairs at random, giving gametes in the proportions 1 AA : 4 Aa : 1 aa; and Gregory's data gave experimental evidence that this method of segregation occurred. The data of Blakeslee, Belling and Farnham on the Jimson weed have since given abundant proof of its application there.[5] Applied to the X's and Y's this would result in 1 XX : 4 XY : 1 YY gamete. It is likely, however, that, on

[4] Muller, H. J., 1914, "A new mode of segregation in Gregory's tetraploid Primulas," AMER. NAT., *48*, p. 508–512.

[5] Blakeslee, A. F., John Belling, and M. E. Farnham, 1920, "Chromosomal duplication and Mendelian phenomena in Datura mutants," *Science*, p. 388–390.

account of the great genetic difference usually existing between the X and Y, the two X's would tend to synapse with each other more closely than with the Y's, which likewise would tend to form a closer pair with each other than with the X's. There is also some experimental basis for this conclusion, as in non-disjunctional diploid females of Drosophila having 2 X's and a Y, the X's have been shown genetically to have a greater tendency to synapse with each other than with the single Y there.[6] In the tetraploid, in the cells in which the X's and Y's paired off more closely in this fashion, like with like, they would tend to segregate accordingly at the reduction division, like from like. Such reduction would give rise to XY gametes. Hence we see that there would be a tendency for tetraploids to form an even greater proportion of XY as compared with XX and YY gametes than shown in the above 1 : 4 : 1 ratio, which is based on random synapsis. Certainly the XY gametes of the tetraploids would be in the great majority.

Now whenever one of the XY gametes fertilized an egg (XX) from a tetraploid, a sterile zygote having an interproportion of sex-producing genes normal to neither male nor female would result. Only a small fraction of the offspring of a tetraploid, then, would be fertile males and females, and hence "reproductive selection" should soon exterminate the tetraploid lines in competition with the freely reproducing diploids—unless, through some strange chance, the tetraploids were favored by reason of some highly advantageous compensating character not present in the diploids, or were somehow removed from competition with the latter.

(4) The only way in which the tetraploid might be freed from the above reproductive handicap would be through the fusion of like sex chromosomes into single masses of double size and "potency," or through the formation of some temporary attachment or attraction

[6] Bridges, C. B., 1916, "Non-disjunction as a proof of the chromosome theory of heredity," *Genetics, 1*, p. 1–52, 107–163.

between them, operative at the reduction division in such manner as to carry like chromosomes to the same pole. A fusion of the X's of the kind discovered by L. V. Morgan[7] in Drosophila would meet the requirements. The origination of such changes in the chromosomes is, however, an exceedingly rare event, if we may judge by the rarity with which it has been discovered in genetic work. And, in order to be of advantage in the reproduction of tetraploids, this fusion or peculiar attraction would have to originate practically simultaneously with tetraploidy. It could not originate much later than the tetraploidy, and still be of use, because, without such an arrangement, the tetraploids would soon have died out. On the other hand, if the fusion or attraction had originated much before the tetraploidy it would have become extinct prior to the occurrence of the latter. This is because an attachment between like sex chromosomes in diploids puts the line containing it to a reproductive disadvantage somewhat similar to that caused by non-attachment of sex chromosomes in tetraploids. For, as in the case of L. V. Morgan's Drosophila, half the offspring of such diploids are infertile or inviable—namely, the 2-X eggs fertilized by X-containing sperm and the no-X eggs fertilized by Y-containing sperm.

It is evident from all the foregoing that a most remarkable concatenation of events must obtain before a persistent tetraploid line can actually become established, and capable of surviving in a state of nature, in animals having the prevalent type of sex determination. Amongst most higher plants, however, the above mentioned difficulties do not apply.

If the present theory is correct, it may receive two lines of support from comparative cytology. For amongst groups of plants the sporophytes of which are always dioecious, and have the "XY" or "WZ" type of sex determination, there should be the same lack of evidence

[7] Morgan, L. V., 1922, "Non-criss-cross inheritance in *Drosophila melanogaster*," *Biol. Bull.*, *42*, p. 267–274.

of tetraploidy as amongst most animals. And amongst groups of animals like the earthworms and fresh-water snails, which are normally hermaphroditic, tetraploidy or even higher forms of polyploidy might occur as readily as amongst most plants. Other things being equal, of course, the more ancient, abundant and diversified the group, the greater should be the chances of finding species which contained chromosome multiples of other species; hence such a test might only be effective in the case of large animal and plant groups. While in some of these it might be that certain peculiar physiological conditions —*e.g.*, the previous attainment of an optimum surface-volume ratio—might render polyploidy disadvantageous, yet in some groups at least it should occur.

In addition to the hermaphroditic groups of animals some of those bisexual groups in which sex is not determined either by sex-gene ratios or by a haploid-diploid relationship should also show polyploidy. Amongst the latter types may perhaps come such forms as the hymenoptera and the rotifers. For, although the male is commonly "haploid," the female "diploid," in these animals, it is gratuitous to assume (as is generally done) that the difference in chromosome quantities is necessarily the sex-differentiating factor here, any more than that a similar difference determines gametophyte versus sporophyte in the moss. As in the latter organisms it has been shown that the determination of sexual versus asexual habit depends upon some "physiological" reaction-complex, set going early in development, and not upon chromosome number, so too in the bee, wasp, etc., it is possible that sex determination depends upon a physiological event decided by the fertilization or non-fertilization of the egg, but independent of chromatin quantity. If this should prove to be true, then these groups also might give evidence of polyploidy.

One important consequence of the prevention of polyploidy in most animals would be a relatively high fixity in number of genes throughout large groups. Another would be the fact that all regions of the chromatin would,

in the course of time, become increasingly differentiated from each other, and incapable of carrying on necessary functions performed by other regions. Hence if only a small section of the chromatin were inactivated or removed from both members of a pair of homologous chromosomes, inviability should result, as in the cases of Bridges'[8] and Mohr's[9] "deficiencies." In plants, on the other hand, where tetraploidy may have occurred in the ancestry fairly recently, from the point of view of evolutionary time, certain pairs of chromosomes would be more apt to be similar to others, and more able to act in substitution for them, than in animals. Here, then, small deficiencies would not so regularly prove lethal, and, in fact, lethal genes as well as "visible mutant genes" should be detectable more rarely (see discussion of tetraploids in paper on balanced lethals[10]). Likewise abnormalities in the numbers of entire chromosomes would have less tendency to produce inviability in plants than in animals, inasmuch as the addition or subtraction of one or more chromosomes from a polyploid-derivative involves a smaller ratio change than a similar addition or subtraction from a true diploid. Besides the above, there should be other differences from animals discernible on exhaustive genetic analysis, such as the existence of certain types of duplicate genes, and their arrangement in similar order in different linkage groups.

It is believed that the arguments for the rarity of tetraploidy in animals rest on a firm foundation of fact, but the discussions of the three preceding paragraphs are admittedly more speculative. They are presented, however, because they are testable. Any useful theory should to some extent be able to predict results in fields in which information is not yet at hand, or at least point the way for further lines of investigation.

[8] Bridges, C. B., 1917, "Deficiency," *Genetics, 2,* p. 445–465.

[9] Mohr, O. L., 1919, "Character changes caused by mutation of an entire region of a chromosome in Drosophila," *Genetics, 4,* p. 275–282.

[10] Muller, H. J., 1918, "Genetic variability, twin hybrids, and constant hybrids in a case of balanced lethal factors," *Genetics, 3,* p. 422–499.

THE ENVIRONMENT OF THE EARLY VERTEBRATES

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AN interesting hypothesis contends that the early vertebrates originated in streams because of their metamerism and fusiform shape, which could only be acquired by organisms dwelling in an environment that constantly moved in one direction.[1]

This hypothesis emphasizes the dynamic condition of flow and assumes that the early vertebrates were static. It seems never to have occurred to the author of this remarkable proposition that the dynamic action of such an environment on static objects finds a natural reciprocal and one which is not only much more readily envisaged but one which agrees far better with the known facts if it be considered that the evolving organism was dynamic and the medium was static. In fact under no other than these latter conditions could the ancestors of our present-day stream biota have developed the proper form and locomotive ability that would permit them to have gained access to a stream environment; nor could they, without it, succeed in maintaining themselves in such an environment, which ever tended to carry them down to the sea.

The mere fact that only a few swimming, creeping, clinging and sessile things from among the vast hosts of marine organisms have succeeded in effecting a permanent invasion of stream waters in the millions of years since they first appeared on the stage of life is readily understandable on this basis. Stream action not only explains the absence of the stocks which have never been

[1] Chamberlin, T. C., "On the habitat of the early vertebrates," *Jour. Geol.*, Vol. 8, pp. 400–412, 1900.

able to take advantage of the age-long opportunity for the invasion of fresh waters, but demands an ability to cope with flowing water from the very beginning of the invasion of such a habitat.

It would not be difficult to imagine a course of evolution conditioned on a dynamic environment in the case of more or less elongated organisms firmly attached by one end of the body, but it is somewhat puzzling to picture any more or less freely moving organism being able to attain and remain in such a stimulating environment while these profound changes in body structure and form were taking place.

It is only just to state that such an inverted picture of evolution has never in so far as I know, been held by any competent ichthyologist. Nevertheless its enthusiastic approval by Grabau[2] in geological, and by Lull[3] in evolutionary texts since it was originally proposed by Chamberlin, seems to warrant an examination of its lack of basis in either fact or theory.

The typical form of the body in the fishes and in fact in other rapidly moving aquatic organisms is roughly fusiform, widest in front of the middle and slightly higher than wide. So common is this shape that Dean estimates that about half the existing fishes approximate this form. From it have probably been derived the dashing type as exemplified by the pickerel, the compressed type like the sunfish, and the depressed type like that of the angler or ray, all adapted for special habits of life.

The typical fish form, often called the stream-line form, does not imply that flowing water had anything to do with its origin. As a dynamical problem of perfecting a shape which would slip through the water with least resistance and without the production of eddies which are the chief factors in retarding progress, it is one that the fishes have solved even better than have the designers of torpedos and submarines.

[2] Grabau, A. W., "Principles of Stratigraphy," Chapter 26, 1913.
[3] Lull, R. S., "Organic Evolution," pp. 462–465, 1917.

Nor are the fishes the only organisms that have perfected such a body form. Among invertebrates it is exemplified in the squids, which no one can doubt are of marine origin; in fact, much of their history is known and can be definitely correlated with their rapidity of movement through the medium and is not attributable to the movement of the medium. It must be obvious that any organism endowed with a forward motion brings about an environmental stimulus of exactly the kind which Professor Chamberlin can find only in streams. And that the former was the stimulus to metamerism as well as bodily form in the fishes and their ancestors it seems to me needs but to be stated to be accepted.

The squid propelled by an excurrent stream of water demands a stiffened axis, hence the retention of vestiges of the ancestral exoskeleton as the internal pen. A chordate like Amphioxus requires a flexible axis, hence the notochord. We are on safe ground in regarding the form and metamerism of the lancelet as giving a fair indication of the body form and habitat of the earliest fishes. That the various existing species of Amphioxus are rather sedentary in habit I regard as a protective adaptation and not as an indication that such a sedentary existence was the habit of the generalized ancestral stock of the vertebrates. Indeed, the metamerism of the lancelet proves that such was not the case.

This metamerism, which is a more fundamental part of the stream origin hypothesis than is the body form, and whose initiation and development is sought in the poetical simile of the waving of the submerged vegetation in a stream or the fluttering of a flag in the breeze, certainly preceded the beginnings of typical fish form. It is found in both marine worms and chordates, and it was unquestionably perfected in some of the chordates, and already present in slow moving eel-like forms, such as the lamprey. As Dean has shown, the simple metamerism of a type like the lamprey may readily be used as the starting point for deriving the more specialized meta-

merism which characterizes the different types of fishes as we know them to-day.

There are a number of collateral arguments that have been advanced, more or less tentatively, it is true, as tending to support the Chamberlin hypothesis. One of these is the peculiarity of the geological record of the earlier known fishes in that their fossil remains are infrequent except in certain deposits like the so-called passage beds between Silurian and Devonian, about which much difference of opinion has arisen as to the precise environment which they indicate.

Purely negative evidence is, of course, of little weight, but there are certain well-known considerations bearing on the absence as well as the presence of fishes in various parts of the geological column that have an interest in the present connection. The primitive fishes had a cartilaginous skeleton and the earlier Paleozoic fish-like vertebrates which figure so largely in the preserved record —the ostracoderms—are forms which developed an exoskeleton. It is this exoskeleton, or the dermal plates and spines of which it was composed, which constitute the bulk of the early records.

The ostracoderms are inconceivable as illustrations of the ancestral type of the true fishes and they are best regarded as early specializations adapted, more or less, to a bottom dwelling and grubbing existence. They certainly lack, for the most part, a body form adapted to swift movement or the capacity for maintaining themselves in currents of any velocity, except as bottom dwellers. The oldest known ostracoderms are those of the middle Ordovician and these occur in sediments of marine origin. Objection may be raised that stream dwellers frequently are carried, either alive or dead, to the sea margin and preserved as fossils in marine deposits, which consideration would perhaps conform to the sporadic occurrence of these forms in the earlier rocks.

That it really matters little in the present case is due to the fact that biologists are agreed that the ostraco-

derms as we know them in the rocks are highly specialized, that their origin must be projected backward to times long anterior to their appearance in the geological record, that they were derived from fish-like forms without durable parts and with presumably a more typical fish-like form, and that they are a sideline without issue and not in the direct line of ascent of the true fishes.

The other cul-de-sac of early vertebrate evolution, the Arthrodira, are much later to appear in the record, being confined to the Devonian and early Mississippian (Lower Carboniferous). Although their internal skeleton is sometimes superficially calcified, they owe their prominence in the geological record to the fact that, like the ostracoderms, they developed an armored exoskeleton. In the relatively abundant fish faunas of the Devonian, especially as exemplified in the Old Red Sandstone, now generally admitted as a series of predominantly continental deposits, we can see traces of adaptive departures from the normal fish form, showing that this was acquired long prior to the Devonian. For example, among the Dipnoi, *Dipterus* of the lower Devonian is much more fishlike than *Phaneropleuron* of the upper Devonian. The latter is more fishlike in body form than *Uronemus* of the Lower Carboniferous, and the last is much less retrogressive than the eel-like existing *Ceratodus* or *Lepidosiren,* as Dollo has pointed out.

In later geological times, with the more complete calcification of the skeleton, as exemplified by the so-called ganoid and teleost fishes we find an abundance of fishes in the rocks, so that we are forced to believe that the geological record is just what might be predicted if the earliest fishes were cartilaginous as all students of fishes believe. Only those with dermal armor are preserved in the earlier rocks, there being no trace of their cartilaginous ancestors or contemporaries. With the progressive calcification of the endoskeleton a relative abundance of fishes is found in the later marine sediments. With these considerations in mind I can not see that the spo-

radic occurrence of fishlike remains in the early Paleozoic has any bearing on the question at issue, for there might just as well have been millions of fishes in both land and marine waters in the early Ordovician and if they had no hard parts, both records would be equally barren.

A consideration of the fitness of the environment furnishes weighty *a priori* considerations for regarding the ocean as having furnished maximum facilities for the origin and evolution of life. Moreover, land waters are from the standpoint of geological time relatively evanescent features and for that reason can have played but a small part as theaters of evolution. The part of the earth's surface which at the present time supports the most abundant and varied population (if the geologically late Hexapoda be excepted) is the pelagic and sublittoral regions of the sea, and there is not the slightest reason to doubt that this has always been the case. The highly varied and swarming arthropod and brachiopod life of the Cambrian was, so far as we know, entirely marine. That so dominant a group of organisms as the trilobites did not invade terrestrial waters, if indeed they did not, for the evidence is of a negative sort and therefore not worth much, was due entirely, I believe, to dearth of food in fresh waters and not because they were dependent on a constant degree of salinity of the water.

In so far as we can judge from the sole survivor of the Meristomata (Limulus), or the existing Crustacea, they are to a very considerable degree oblivious to the degree of salinity of their environment so long as food is plentiful and the other environmental factors are not prohibitive. Many regard the primitive oceans as having been practically free from salts, and although there is considerable geophysical ground for such an opinion, such a condition was remotely anterior to that part of the past which is concerned with the origin of fishes, since the evidence of marine organisms with hard parts in the early Paleozoic and even in the pre-Paleozoic shows clearly that, if such was true of primordial ocean waters, that

condition had long since been modified. Indeed, the evidence of the time involved and the extensive weathering of the pre-Cambrian rocks would in itself be sufficient proof of the probability of my statement.

The relative sparsity of organisms in the fresh waters of the globe and the variety of marine types which have never colonized these waters is one of the remarkable facts of biology. I regard the lack of permanence of fresh waters and their relatively poverty in food resources as a consideration entitled to great weight in any consideration of the place of origin of fishes or eurypterids. And if the fresh waters of the globe are to-day relatively poor in foodstuffs, how much poorer were they in the far distant Paleozoic before there were insects on the land or before the Mollusca or Crustacea or higher worms had invaded fresh waters. If the dominant early Paleozoic trilobites invaded the land as many students have supposed, it was probably by way of the strand, as did the Isopod Crustacea at a much later date, and not by way of fresh water. In fact, if there were no good arguments to be derived from other sources I would regard the foregoing considerations as amply nullifying the hypotheses that either the eurypterids or the fishes were fresh-water evolutionary products.

If the reader thinks of freshwater vegetation as a source of food it should be pointed out that we know casts of the alimentary canals of both trilobites and eurypterids and in both cases it is small and straight and indicative of a highly concentrated, non-vegetable diet.

Another possible argument has been based on the singular migratory habits of certain marine fishes, notably the various species of salmon and the shad, which have been shown by tagging experiments to return, often great distances, to spawn in the very stream in which they were hatched. Whether this is true of all fishes which enter streams to propagate is not known, but it has been suggested by Chamberlin and others that this might be considered as an indication of their return to the ancestral home of the race.

This homing instinct is also exhibited by the migratory birds and may be universally true of migratory animals, but that it means that the migratory birds originated in the region where they nest and rear their young is vigorously disputed by Brooks. It seems to me that it is about as logical and convincing as the explanation given by Crotch for the occasional migration of lemmings in Scandinavia, where those that survive the perils of the journey from the mountains across the lowlands are eventually drowned in the sea, which, Crotch states, they are endeavoring to cross to reach the lost Atlantis which their ancestors inhabited in Miocene times.

We are profoundly ignorant of the real explanation of this remarkable habit, but it may be remarked that it is one absent in many marine fishes and that many more go no farther than coming to the shore at the reproductive season, and at least one type—the eels—pass their mature existence in fresh waters and go far out to sea to spawn, possibly seeking for the rivers of the mythical Atlantis in which their ancestors first saw the light.

Moreover, lake fishes often show a similar habit in ascending tributary streams to spawn, and various lower marine organisms such as the squids and comatulid crinoids show a comparable instinct, coming from the depths into shallow water for reproductive purposes.

Brooks long ago suggested (*Pop. Sci. Monthly,* April, 1898) that migration in both fishes and birds might be considered a result of natural selection in the effort to find a safe place in which to lay eggs and rear young and that the enemies to eggs and young in the case of the fishes would be much fewer in streams than along the shoals of the seacoast. It certainly seems remarkable that such fishes as the shad or salmon, which are especially adapted for their marine habitat, and which apparently take no food during their freshwater journey, should be just the fishes that exhibit this homing instinct in its perfection.

One thinks of many factors, such as oxygen supply or hydrogen ion content or temperature, which might be

correlated with the almost universal *wanderlust* preceding mating that might explain the habit. Meek,[4] who has published much on this subject, feels sure that the upstream tropism is due entirely to the current, and this may be the orientation factor, but it falls far short of explaining the preliminary sea voyage and coasting that precede the river trip.

Certainly unless one is prepared to admit that the salmon has a better racial memory than thousands of other marine fishes, one can not consider its habits as an argument in support of the hypothesis that the early vertebrates originated in streams.

SUMMARY

(1) The form and metamerism of fishes can only be explained as the result of the dynamics of a moving organism and not by the action of a moving medium on a sedentary organism.

(2) The latter could not maintain itself in a moving medium during the time required for its evolution into a form adapted for such an environment.

(3) The form and metamerism as exemplified in the earliest fishes were approximated long previous to the vertebrate stage, and were essential for the initial invasion of the streams by marine ancestors.

(4) The food content of the early streams was insufficient, and their duration in terms of geologic time was too transient, for them to have been centers of evolution.

(5) The geologic record of the fishes conforms exactly to the requirements of the argument, to wit: In the older rocks when the internal skeleton was cartilaginous there are no traces of fishes except a few sporadic occurrences of such as had developed a durable external skeleton: in the later marine rocks when the skeleton had become calcified there are abundant traces of fishes.

(6) The migration of a very small percentage of recent fishes from the oceans to streams for the purpose of spawning is shown to have no relevancy with regard to the original home of the race.

[4] Meek, A., Rept. Dove Marine Lab., No. 6, pp. 52–54, 1917.

SHORTER ARTICLES AND DISCUSSION

CORRELATION AND MACHINE CALCULATION

At various times during the past few months there have been rumors of wonderful new methods of determining correlation by the use of modern commercial machines permitting simultaneous multiplication and summation. The results now appear in a bulletin by Wallace and Snedecor.[1]

The need for quantitative work in biology is so urgent and the labor of adequate statistical analysis is so great that it may seem ungracious to imply any criticism of any discussion of methods of calculation which may be of use. There is, however, always a real danger that younger students, in accepting as wholly new methods which have long been known to biometrists, will fail to go to the original sources where the methods were first described and provided with illustrations of applicability.

About twenty years ago I recognized clearly the need for the development of formulae which would permit (*a*) the wider use of mechanical computing equipment, and (*b*) the organization of the routine work in such a manner as to attain the maximum number of end results and provide the maximum number of checks for the work with the minimum of arithmetical effort. The results have been given in a number of papers, the first three of which were published in this journal.

While I have assiduously practiced the scientific virtue of citing these papers of my own on every possible occasion, including both review[2] and criticism,[3] they do not seem to have come to the attention of the authors of "Correlation and Machine Calculation." That they have been used at all is perhaps largely due to the fact that I have personally taught at least a hundred biologists the advantage of one or more of these methods. In view of the fact that Wallace and Snedecor give only a very incomplete account of the whole problem of machine calculation, a brief reference to the literature may be helpful.

[1] Wallace, H. A., and Snedecor, W., "Correlation and Machine Calculation," Off. Pub. Iowa St. Col. Agr. Mech. Arts, Vol. 23, No. 35, 47 pp. 1925.

[2] Harris, J. Arthur, "An outline of current progress in the theory of correlation and contingency," Amer. Nat. **50**: 53–64, 1916.

[3] Harris, J. Arthur, "Reed on the coefficient of correlation," Quart. Pub. Amer. Stat. Ass. **15**: 803–805, 1917.

The first and only essential for the use of modern computing and tabulating equipment in the rapid calculation of statistical constants is the calculation of the moments in terms of the actual measurements instead of in terms of deviations of these measurements from the true mean, or about some value selected arbitrarily but in a way to reduce arithmetical effort. As far as I am aware methods of doing this mechanically for the determination of the first several moments of frequency distributions were first given long ago by Hardy in the graduation of the British Office Life Tables. Hardy's method was subsequently described and elaborated by Elderton[4] (p. 19–23), and by Hardy himself.[5] It seems never to have been adopted in biological work, although it presents certain advantages when equipment adopted primarily for addition only is available. Hardy's method has the disadvantage that the moments are really taken about an arbitrary origin. This precludes the development of any general system of formulae.

In 1910 I pointed out the many advantages of taking the moments about 0 as origin. This is the essence of the method now described by Wallace and Snedecor for the determination of r. In this paper[6] the advantages of the method for raw and tabulated data, for the securing of checks on the accuracy of the arithmetical work, for the calculation of means of arrays for regression tests and for the calculation of the correlation ratio are fully set forth. Immediately afterwards essentially the same methods were applied to the formation of correlation and contingency tables[7] and of condensed correlation tables[8] in cases in

[4] Elderton, W. P., "Frequency-Curves and Correlations," London (1905).

[5] Hardy, G. F., "The theory of the construction of tables of mortality and of similar statistical tables in use by the actuary," London, 1909. See especially p. 59–63, 124–128.

[6] Harris, J. Arthur, "The arithmetic of the product moment method of calculating the coefficient of correlation," AMER. NAT. **44**: 693–699, 1910. When this paper was written I was not aware that MacDonell had used (p. 231) the same formula for the product moment coefficient in a craniometric investigation published six years earlier. See W. R. Macdonnel, *Biometrika* **3**: 231, 1904. His method, like that of Hardy, seems never to have been used by other biometricians.

[7] Harris, J. Arthur, "On the formation of correlation and contingency tables when the number of combinations is large," AMER. NAT., **45**: 566–571, 1911.

[8] Harris, J. Arthur, "The formation of condensed correlation tables when the number of combinations is large," AMER. NAT., **46**: 477–486, 1912.

which the number of possible combinations is large. These served as a basis for the development of a fairly complete system of formulae for the calculation of intra-class and inter-class coefficients from class moments under similar conditions.[9] This theory was the immediate basis for the suggestion of a substratum heterogeneity coefficient[10] which has been extensively applied in the analysis of agronomic experiments.[11] The possibility of spurious correlation due to disorderly differentiation in the use of such formulae was early recognized.[12]

Formulae for the calculation of the correlation between growth increments have been developed,[13] illustrated[14] and utilized in determining the probable error of the difference between two differences,[15] as illustrated elsewhere.[16]

Formulae for the correlation between a component, and between the sum of two or more components, and the sum of the remaining components of a variable have also been given[17] in terms of moments about 0 as origin.

The method of moments about 0 as origin was utilized in the development of a portion of the theory of correlation between a variable and the division of a dependent variable from its probable value, particularly in determining the standard deviation

[9] Harris, J. Arthur, "On the calculation of intra-class and inter-class coefficients of correlation from class moments when the number of possible combinations is large," *Biometrika*, 9: 446–472, 1913.

[10] Harris, J. Arthur, "On a criterion of substratum homogeneity (or heterogeneity) in field experiments," AMER. NAT., 49: 430–454, 1915.

[11] Harris, J. Arthur, "Practical universality of field heterogeneity as a factor influencing plot yields," *Jour. Agr. Res.*, 19: 279–314, 1920.

[12] Harris, J. Arthur, "On spurious values of intra-class correlation coefficients arising from disorderly differentiation within the classes," *Biometrika*, 10: 412–416, 1914.

[13] Harris, J. Arthur, "Formulae for the determination of the correlations of size and of growth increments in the developing organisms," Proc. Soc. Exp. Biol. and Med., 18: 4–5, 1921.

[14] Harris, J. Arthur, and Reed, H. S., "Inter-periodic correlation in the analysis of growth," *Biol. Bull.*, 40: 243–258, 1921.

[15] Harris, J. Arthur, "The probable error of the difference between two differences," *Jour. Amer. Statis. Assoc.*, 18: 514, 1922.

[16] Harris, J. Arthur, Lawrence, Z. W., Hoffman, W. F., Lawrence, J. V., and Valentine, A. T., "The tissue fluids of Egyptian and Upland cotton and their F_1 hybrid," *Jour. Agr. Res.*, 27: 267–328, 1924.

[17] Harris, J. Arthur, "The correlation between a component, and between the sum of two or more components, and the sum of the remaining components of a variable," Quart. Pub. Amer. Statis. Assoc., 15: 854–859, 1917.

of the deviation of the dependent variable from its probable value and its regression on the independent variable.[18] It has also been used in determining the correlation between a variable and the deviation of an associated but not dependent variable from its probable value.[19]

These papers owe their origin primarily to a realization of the need of replacing mental and manual work by purely mechanical methods. All give formulae and methods which may be useful to those interested in the surprisingly new idea of "correlation and machine calculation."

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VARIATION IN THE INTENSITY OF LINKAGE IN MAIZE

LINKED endosperm characters in maize provide excellent material for the study of problems of linkage, for each well-developed ear comprises a population of hundreds of individuals, and segregation ratios may be determined in the season in which pollinations are made. One known linkage group includes at least four genes affecting endosperm characters: *C c* and *I i*, which in the presence of certain other genes determine aleurone color; *Sh sh*, which affects the degree of shrinkage at maturity; and *Wx wx*, which affects the chemical composition. Studies of linkage in this group have been reported by Collins and Kempton[1] and by Hutchinson.[2]

Since the complementary action of *C* and *i i* is essential to the production of aleurone color, segregation for these two genes can not be studied in the same material. But by the use of appropriate material it is possible to determine simultaneously

[18] Harris, J. Arthur, "Further illustrations of the applicability of a coefficient measuring the correlation between a variable and the deviation of a dependent variable from its probable value," *Genetics*, **3**: 328–352, 1918.

[19] Harris, J. Arthur, "On the correlation between a variable and the deviation of an associated but not dependent variable from its probable value," *Jour. Amer. Statis. Assoc.*, **18**: 393–394, 1922.

[1] IV. Conf. Int. Gen., pp. 347–356 (1911); AM. NAT., 46, pp. 569–590 (1912); U. S. Dept. Agr. Dept. Bul. 754 (1919).

[2] *Jour. Heredity*, 12, pp. 76–83 (1921); Cornell Agr. Expt. Sta. Mem. 60 (1922).

the linkage relations of *C c* (or *I i*), *Sh sh* and *Wx wx*. I am indebted to Dr. R. A. Emerson for supplying me with such material, which made possible the study here reported. This material was the seed of three ears, two of them heterozygous for *C c* and *Wx wx*, and the third heterozygous for *C c*, *Sh sh* and *Wx wx*, together with appropriate multiple recessive stocks for backcrossing. All the stocks are homozygous for *A A*, *R R* and *i i*, thus permitting the identification of *c c* by means of aleurone color.

Significant differences in crossing-over in Drosophila, dependent on both environmental and genetic factors, have been shown in a number of recent investigations. Bridges[3] found that second brood progenies (from eggs laid during the second 10-day period) showed a significantly lower crossover percentage for certain genes of the second chromosome than did progenies of the first brood. Plough[4] obtained striking differences in crossover percentages in the second chromosome and in a portion of the third chromosome under different temperatures, the percentage of crossing-over being greatly increased by extreme temperatures both high and low. No difference in crossing-over resulted from temperature extremes in the remainder of the third chromosome or in the sex chromosome. The regions showing variation in crossover percentage under temperature differences coincided with those showing variation with age. Recently, Mavor[5] has been able to produce marked changes in crossover values by X-ray treatments, both in the sex chromosome and the second chromosome, crossover percentages being decreased in the sex chromosome and increased in the second chromosome by similar treatment.

Genetic differences in linkage intensity in Drosophila have been reported by Sturtevant[6] and Detlefsen.[7] Sturtevant found crossover percentages in the second chromosome greatly reduced in a strain of flies derived from a wild-type female from Nova Scotia. He demonstrated the existence of two genes affecting the amount of crossing-over in the second chromosome. Detlefsen was able by continued selection to reduce greatly the cross-

[3] *Jour. Expt. Zool.*, 19, pp. 1–21 (1915).

[4] *Jour. Expt. Zool.*, 24, pp. 147–209 (1917); 32, pp. 187–202 (1921).

[5] *Science*, 58, pp. 124–126 (1923); Proc. Soc. Expt. Biol. and Med., 20, pp. 335–338 (1923).

[6] Carnegie Inst., Washington, Publ. 278, pp. 305–341 (1919).

[7] Proc. Nat. Acad. Sci., 6, pp. 663–670 (1920); 9, pp. 149–156 (1923).

over percentage in the sex chromosome. In crosses between high crossover and low crossover flies thus produced, crossover values were more variable and consistently higher in the F_2 than in the F_1 generation.

No direct investigations of germinal or environmental variation in linkage intensity in plants have been reported, to my knowledge, though a number of investigators have studied the relative crossover percentage in male and female gametogenesis. In discussing some apparently significant differences in crossover percentage in microsporogenesis and megasporogenesis in maize, Emerson and Hutchinson[8] point out that these may be due to disturbing conditions, such as temperature effects, rather than to inherent differences in the mechanism of crossing-over. Hutchinson[2] found crossing-over between *Sh* and *Wx* materially higher in his *I–Sh–Wx* stock than in his *C–Sh–Wx* stock, and suggested that possibly a crossover modifier such as that described by Sturtevant might be responsible.

The present investigation is planned as a study of genetic and environmental variation in crossing-over in maize. During 1923 the production of seed for future use was the chief concern, but it was possible incidentally to make a number of backcrosses for linkage data, using the heterozygous plants as seed parents. Data on crossing-over in the *C–Wx* region are available from 115 plants, including something over 50,000 grains. These data give some rather interesting indications which are the subject of this note.

The percentage of crossovers differed markedly in the three families, as indicated in the table below:

Family	Number of plants	Number of grains		Crossover percentage
		total	crossovers	
A	51	25,757	5,326	17.90 ± 0.15
B	20	8,308	1,879	22.62 ± 0.31
C	29	8,446	2,183	25.85 ± 0.32

The difference between families A and B is 4.72 ± 0.34, between families B and C 3.23 ± 0.45, and between families A and C 7.95 ± 0.35. All three differences are statistically significant according to any ordinary standard.

The crossover percentages and probable errors given above are computed in the usual manner from the total number of cross-

[8] *Genetics*, 6, pp. 417–432 (1921).

overs and the total number of individuals in each group. They thus represent the results which would be obtained from determinations of the percentage of crossing-over between *C* and *Wx* in the three families independently. The probable errors take no account of the variation between the plants within the group —they are dependent only on the average crossover percentage and the total number of individuals (grains) within the group. Considering each family as a random sample from a population of plants varying in crossover percentage, the differences between the families might result simply from error of sampling. The data have therefore been reworked from this standpoint, the crossover percentages for individual plants in each family being averaged and the probable errors of their means determined by Bessel's formula. The results are as follows:

Family	Mean crossover percentage
A	18.14 ± 0.26
B	22.47 ± 0.47
C	25.99 ± 0.43

The differences are as follows: between A and B 4.33 ± 0.54 per cent., between B and C 3.52 ± 0.64 per cent., between A and C 7.85 ± 0.50 per cent. Although the probable errors have been increased in all cases, all the differences remain clearly significant.

It is of course possible that different environmental conditions during the maturation period may account for these differences. The flowering dates, which were noted for each plant, averaged about two days later in family B than in family A, and about three days later in family C than in family B. But fifteen plants of family A, from a planting two weeks later (flowering dates about 8 days late) gave an average crossover percentage of 20.11 ± 0.68, which is not significantly different from that of the first planting of family A, as given above. Moreover, no relation of flowering dates to crossover percentages was apparent within any of the families.

Environmental variation in linkage was indicated by the relatively wide differences in crossover percentage determined from different ears of the same plant. If these differences are due only to error of sampling differences greater than their probable errors should occur in only about one half of the cases, and the expected frequency of differences of various multiples of the

probable error can easily be determined. Comparisons of crossover percentages of different ears of the same plant may be made in 47 cases in family A (first planting).

The occurrence of differences of various extent in comparison with their probable errors, together with the number expected if the differences were due entirely to error of sampling, are shown in the following table:

Difference / P. E.	Probable occurrence in 47 trials through error of sampling	Observed occurrence
1.0 or more	23.5	29
1.5 or more	14.7	24
2.0 or more	8.3	18
2.5 or more	4.3	11
3.0 or more	2.0	7
3.5 or more	0.9	5
4.0 or more	0.3	2
4.5 or more	0.1	1

The rather wide differences in crossing-over between ears of the same plant suggest that significant differences in crossover percentage in megasporogenesis and microsporogenesis, such as those reported by Emerson and Hutchinson, may be due merely to different environmental conditions during the maturation of male and female gametes, a possibility which was suggested by those authors.

Of the 33 plants in family A in which the crossover percentage for the first and second ear was determined, 20 gave a higher percentage in the first ear, but the average difference was less than 1 per cent. and was not statistically significant.

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THE HOST-PARASITE METHOD AND THE DISTRIBUTION OF FROGS

IN the March-April number of the AMERICAN NATURALIST for 1923 I attempted to apply the well-known dispersal theses of Matthew to the Amphibians. In dealing with the *Salientia* I came to no new or striking conclusions, as I utilized the recent classificatory work of my friend Noble, and my notions of their dispersal were quite in accord with his.

Metcalf, in his work on the *Opalinidae*, came to quite different conclusions regarding the dispersal of their hosts, and has recently reiterated these conclusions in order to refute my statements.

Many elements enter into the discussion of this matter, and conclusions as to dispersal are the final results drawn from consideration of certain facts and from the interpretation of those facts.

The first set of facts to be considered is the whole mass of data regarding the anatomy, phylogeny, distribution and classification of the *Opalinidae*. These facts have been discovered and set forth by Metcalf and I find myself perfectly willing to accept them at their face value.

The second set of facts to be considered is the similar data regarding the anatomy, phylogeny, distribution and classification of the *Salientia*. Here, of course, the matter becomes herpetological, and many of Metcalf's statements are open to attack.

Third, there is the question as to whether these two sets of facts coincide, for upon this coincidence of the classification and distribution of the two groups hangs the right of the parasitologist to discuss anything regarding the hosts.

Fourth, even if this coincidence is found to exist, there remains the question as to its meaning and its applicability to discussions of dispersal.

Regarding the first set of facts, I have nothing to say. Regarding the second I find many statements by Metcalf with which I am compelled to disagree. I hasten to admit that most of these herpetological pronunciamentos to which I object have no bearing on the argument, as, for instance, *Liopelma*, the New Zealand frog, which is, according to Metcalf, a Leptodactylid. This frog is absolutely known to be a Discoglossid, yet, as its parasites are unknown, there is no reason for its inclusion in the present discussion.

The recognition of the *Brachycephalidae* as a natural family, which Metcalf denies, would really strengthen his case, for their parasites are not those of the *Ranidae*, with which they are usually associated in the classifications, but rather the parasites of the *"Leptodactylidae"* from which, according to Noble, the *Brachycephalidae* have arisen.

Still more remarkable and more pertinent is his statement that the families *Ranidae* and *Hylidae* do not compete because of

their different habits, and that therefore there is no reason for the absence of *Hyla* in Africa and in the Oriental region. This statement is made in the face of the well-known and elaborate arboreal adaptations of Ranids in these very regions, facts which have often been used to explain the absence of Hylids.

More to the point, however, is the question of the coincidence of data drawn from both host and parasite. 38 genera and 163 species of frogs have been found parasitized by *Opalinidae*, and upon this rather slender sample of the Salientian fauna of the world hangs Metcalf's whole scheme of Salientian dispersal. There is little specific infection noticeable. 41 of the 163 species of frogs are known to harbor more than one species of parasite, and 14 species of Opalinids infect more than one host. Nor is the case much better when we examine genera rather than species. Genera are *not* marked by uniformity of parasite. In only five of the 38 genera have five or more species been found parasitized. These are *Bufo, Hyla, Rana, Scaphiopus* and *Leptodactylus.* There are four genera of *Opalinidae. All four* of these infect each of the genera *Bufo, Hyla, Rana* and *Scaphiopus. Leptodactylus* alone of the genera in which five or more species have been found parasitized is infected by a single genus of parasite, *Zelleriella.*

When one inquires into this situation, so unfortunate for the host-parasite method, one is forced to surmise that it is barely possible that the parasites themselves have their own distribution which does not altogether correspond with the distribution of their hosts. Thus, in the genus *Hyla,* so far as known from the 23 species found parasitized, the Hylas of Australia have *Protoopalina,* in North America and in Eurasia they have *Opalina,* whereas in South America they have *Cepedea* and *Zelleriella,* with an occasional *Opalina.* Here is a complete lack of that coincidence of the classification and distribution of the two groups upon which hangs the claim of the parasitologist to discuss the dispersal of the host. And the distribution of the genus *Hyla* is one of the great puzzles of herpetology which the host-parasite method is so suited to solve!

Even if this coincidence is proven to exist, and it seems somewhat sadly to seek, it has been taken by Metcalf for much more than its face value. I can not regard the host-parasite method with the proper awe. It is a valuable check and nothing more. And it is a check on the *classification* of the hosts, not on their

dispersal. We have to explain discontinuous distribution of the hosts. In any such case there are two possibilities: either the classification of the hosts is erroneous and the two components form an unnatural assemblage of forms, or the classification of the hosts is correct and the two components form a natural assemblage of forms and a once continuous distribution has become discontinuous. The host parasite method may help us to decide which of these two propositions is true. Beyond this it is not only valueless but misleading, for if the classification of host and parasite agree our hearts are a bit more strongly inclined to believe that we are dealing with a natural group whose distribution has become discontinuous, and we are where we started, even though somewhat more firmly fixed in our original position. *Any* theory of dispersal which will enable the host to arrive at its present estate will obviously serve equally well for the parasite which accompanies the host on its peregrinations.

Let us now pray humbly that light may be vouchsafed us and examine two of the outstanding anomalies in frog distribution upon which we have the new parasite data.

The first of these is the presence of *Hyla* in Australia and in South America in numbers, its relative scantiness in the Holarctic region, and its rarity or non-existence in Africa and in Indo-Malaysia.

The Hylas of the northern world have *Opalina,* the Australians *Protoopalina,* and the South Americans have *Cepedea* and *Zelleriella,* with occasionally *Opalina.* Here there is little to enlighten us. Additional evidence that the Eurasiatic Hylas are related to North American forms. *Perhaps* additional evidence that Southern Hylas are more primitive than Northern, since *Opalina* is the most specialized genus. No evidence from parasitology that Australian and South American Hylids are closely allied. No *additional* evidence from parasitology as to the route by which the *Hylidae* have reached their present homes.

It would be very interesting to know the parasite of the Abyssinian *Hyla wachei,* which is apparently allied to South American forms.

The second anomaly is the presence of toothed Bufonids (Leptodactylids) in South and Central America and in Australia and their absence from the rest of the world. The toothed Bufonid of South Africa, *Heleophryne,* is not admitted to the discussion, as its parasites are unknown. The facts in the present instance are that the toothed Bufonids of Australia and South America

are both infected by *Zelleriella* and by *Protoopalina*. *Zelleriella* is found only in Australia and in the New World. Here is indeed that happy coincidence of the classification and distribution of the two groups for which we sought in vain in the case of the similarly distributed *Hylidae*. Here is the prize exhibit, and what does it prove? It is *additional* evidence that the toothed Bufonids are a natural group. No evidence whatever as to the routes whereby these Zelleriella-bitten Bufonids arrived in Brazil and Botany Bay. We are left as much in the dark as to the travels of Salientia as we were before the advent of this *lucus a non lucendo*. And this does not even take into account the fact that arboreal Ranids may easily be the cause of the general disappearance of Hylids from Africa and from Indo-Malaysia; or that the single *Hyla* and single-toothed Bufonid in Africa require a whole additional set of coincidental infections to explain them rather than the general excommunication which Metcalf has pronounced against them because their parasites are unknown. Indeed, these two African cases go far to render the triradial northern dispersal the more attractive theory and incline our hearts more and more to relegate the whole Bifrostean hypothesis to the domain of mythology, whence came Atlantis and St. Brandan's Isle and Tir-nan-oge.

These two examples of Hylids and toothed Bufonids are in no way explained by the additional data derived from their Opalinid parasites. And yet these two cases are the cornerstone of the whole edifice, the keystone of the landbridge so airily cast across the longest east-west stretch of water in the world, the South Pacific Ocean. To this bridge the Opalinids offer no real support. Upon what then are they based? It is the old, old story of the similar fauna of South America and Australia. The old bridge originally erected for the marsupials and fallen down upon the discovery of fossils in the North is now hoisted once more from the Globigerina ooze and serves to transport the poor parasitized Leptodactylids, which were not the cowanderers of the marsupials, because, forsooth, no fossils are known.

The actual argument, then, is as follows:

Marsupials and Leptodactylids have similar distributions.

Marsupials came from the north because we have fossils.

Leptodactylids, on the contrary, came from the south, because we have no northern fossils.

Thus are similar ends attained by different methods, because small creatures like frogs do not make (1) many (2) identifiable fossils.

A final word on "the geographic center of the group range," which is "the center of available migration routes," and which I proposed as perhaps the best method of determining the center of dispersal of a group, inasmuch as the location of the primitive members or the specialized members is not necessarily the center, as Matthew and myself have shown.

This geographic center is eschewed by Metcalf as geometric rather than biologic. Yet it seems to be used by him with less salt than I should have judged possible from the Petrine fervor of his denial; for nothing can be more neatly geometric than the straight and narrow path, built upon the abyssal ooze and connecting South America and Australia; although the more biological qualification, "the center of available migration routes," seems to suffer from neglect.

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FORTUITOUS VARIATION

THE theory of natural selection takes for granted the occurrence of heritable variations in all parts of all organisms. Its effectiveness as a working principle depends on the number and extent of such changes. Observations show that variations are universal in organisms and that they are fortuitous; that is, occur according to chance. According to this view there is no more reason to expect variations to appear in one direction than in another, or that there should be more favorable variations than unfavorable.

All variations, however, can not be classed as favorable or unfavorable. Some occupy a neutral position, neither stimulating the growth and reproduction of the organism nor inhibiting its development and propagation. Such variations do not come under the influence of natural selection, because they are of no selective or survival value. It is of little consequence to the organism whether these variations persist or not, as the organism can live and reproduce as well with them as without them. Color of grain in corn and slight heritable increases or decreases in oil or protein in seeds are examples.

Darwin stated that our ignorance of the causes of variation is profound. This describes the situation as well to-day as it did at the time this statement was made. Lamarck believed that the

direct action of environmental conditions was responsible for the appearance of heritable variations, but this has not been proved. Various attempts have been made to induce mutations by artificial means. To date, however, with one or two possible exceptions, the results have been negative. Only a few years ago there were supporters for the idea that selection, carried on in a definite direction over a period of years, induced heritable changes in that direction. Such changes have now been interpreted in other ways. Furthermore, the character is the manifestation of the factor which precedes the character in ontogeny, and the character must appear, of course, before selection can become operative. Hence, selection can scarcely be said to induce germinal changes when such changes had already taken place in the germ plasm before the organism was subjected to selection.

Whatever may be the cause of variations, it appears certain that they are not induced by a need for them on the part of the organism concerned. In favor of this view is the idea above expressed; namely, that variations occur in characters that are neither favorable nor unfavorable or in degrees that are so slight as to preclude the possibility of their being of any selective or survival value to the organism. Furthermore, there are cases in which variations are known to have occurred prior to the time they are needed to preserve the organism, and hence are already present when needed. This is exemplified in variations enabling the plant to resist fungus diseases and insect pests.

The above comments are suggested by results recently obtained in an experiment on breeding soybeans containing either high or low oil content at the Illinois Agricultural Experiment Station. In 1922 several hundred plants were analyzed separately for oil content. Considerable variation was found. No doubt some of this variation was due to environmental factors, though all plants were grown under as nearly the same soil and climatic conditions as possible. That some of this variation was genetic, however, is indicated by progeny tests. To determine to what extent the extreme variations in oil percentage were inherited a number of the highest and the lowest plants in oil content were selected and progenies grown in 1923. The results are shown in Table I. All the progenies but one from the high parents were very similar in oil content. The exception (Progeny No. 2021) is significantly lower than the rest, even though its parent gave approximately the same analysis as the other high selections. This is but another instance of the effect of the environment in

masking the genetic constitution of an organism. Progenies of the low selections were much less consistent in oil content than those of the high selections. Many of them showed significant differences when compared either with one another or with progenies from the high selections. This is a strong indication

TABLE I

PERCENTAGE OF OIL IN PARENT PLANTS AND PROGENY

	Parent No.	Analysis of Parent	Progeny No.	Mean per cent. oil of progeny	Difference	Difference / Probable error of diff.
High Selections	1590–5	21.23	2018	18.66 ± .0832		
	1590–6	21.52	2019	19.15 ± .1176		
	1590–10	21.35	2021	17.85 ± .2296	1.30 ± .2580	5.04
				15.29 ± .0915		
	1590–16	21.56	2023	18.97 ± .0814		
	1590–17	21.78	2024	19.20 ± .0941		
	1590–19	22.59	2025	19.57 ± .0779		
	1590–22	21.54	2026	18.62 ± .0798		
	1590–28	21.53	2027	19.33 ± .1037		
	1590–35	21.96	2028	19.86 ± .1136		
	1590–43	21.07	2029	19.97 ± .1387		
	1590–44	21.56	2030	19.96 ± .0912		
	1590–57	21.20	2032	19.61 ± .0977		
	1590–62	21.84	2033	20.10 ± .1114		
	1590–65	21.75	2034	19.50 ± .1769		
	1590–69	21.88	2036	19.74 ± .1175		
	1590–77	21.98	2038	20.18 ± .1135		
	1590–84	21.57	2039	20.20 ± .0997		
	1590–89	21.82	2040	19.54 ± .1508		
	1590–91	21.79	2041	19.19 ± .0819		
	1590–92	21.77	2042	19.58 ± .0862		
	1590–136	21.82	2045	20.35 ± .0859		
	1590–171	21.79	2047	19.89 ± .1058		
	1590–175	21.72	2049	20.60 ± .1089		
	1590–191	21.62	2050	20.19 ± .1383		
	1590–197	21.67	2052	18.99 ± .1160		
	1590–204	21.68	2053	20.15 ± .0764		
Low Selections	1590–8	18.33	2020	15.29 ± .0915	2.20 ± .1476	14.91
	1590–67	18.38	2035	17.49 ± .1158		
	1590–72A	18.16	2037	18.10 ± .0787	2.22 ± .1288	17.24
	1590–96	17.99	2043	15.88 ± .1020		
	1590–162	17.82	2046	19.70 ± .0888	3.30 ± .2670	12.36
	1590–172	17.96	2048	16.40 ± .2518		
	1590–195	18.09	2051	17.00 ± .1194		

that some of the variations shown by the parent plants grown in 1922 were heritable. Hence, in this variety, at various times in the past, germinal changes have occurred affecting oil content, and as a result the variety is a mixture of types so far as this character is concerned. The heterogeneous nature of the variety with respect to oil content would never have been detected had analyses not been made.

Similar statements can be made respecting resistance to plant diseases. Heritable variations occur from time to time, irrespective of whether the variety is ever subjected to attacks of the causal organisms or not. As a result, when such attacks occur, individual plants are found which are partially or wholly resistant and hence are able to survive and propagate themselves. In this way resistant varieties are produced. Resistance to plant disease, therefore, is not a variation induced by a need for it, felt by the individual plant; for such a variation occurred prior to such need. *Also, if, after the attacks of the causal organism, further variations occur in the direction of more nearly complete resistance, it seems reasonable to suppose that the same cause or causes which produced the initial variations are operating to bring about further germinal changes, rather than that the result is due to the presence of the causal organism.*

Variations of the type mentioned are physiological rather than morphological. In a few cases disease resistance in plants may be concerned with form or structure, but it is probably, in most cases, bound up with the physiological processes of the plant. Physiological characters are of great importance to the plant in growth and reproduction. The difficulty is that these characters can usually be detected only by performance or behavior rather than by appearance. Heritable variations in such characters are of considerable importance in plant breeding, but such variations are hard to study and fix. Winter hardiness, resistance to lodging, ability to endure keen competition and efficiency of root systems are typical examples of characters which are considered physiological in nature.

It is deemed safe to predict that future work in plant breeding will be concerned more and more with physiological characters. Progress along this line is not hindered by the lack of variations, but rather by inability to detect them. As new and more refined methods are developed it will be possible to isolate lines which excel in this or that physiological character and by combining

these lines through hybridization, to produce types better adapted to specific purposes than those existing at present.

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FERTILITY AND TOLERATION OF TEMPERATURE IN INBRED DROSOPHILA[1]

THE amount of variability with regard to the toleration of temperatures above the normal range exhibited by different wild strains of *Drosophila melanogaster*, reported in a previous paper,[2] suggested the possibility of segregating lines by inbreeding within a single strain which might show similar variations.

With this possibility in mind, a single pair of flies was segregated in January, 1923, from a mass culture of Drosophila collected in Sweden by Dr. G. Bonnier, and three pairs of the first generation offspring were used to start three lines of brother-sister matings. This strain of Drosophila had been kept in the laboratory for the preceding three months and had shown high viability in connection with other experiments. The three lines were kept at room temperature in quarter-pint milk bottles containing the usual banana agar culture medium which has been found to give fairly uniform conditions. The Sweden stock was run simultaneously in mass cultures.

In the fifth inbred generation the "C" line developed an eye mutation[3] which was carried in the line during the experiments. With this exception the four sets ran uniformly, varying only slightly in time of hatching and development until August, when the three lines were in the fourteenth inbred generation. At the time of hatching out the fifteenth generation the lines were at Woods Hole, where they were subjected to considerable temperature fluctuation from the heat of August days to the cool nights.

[1] From the Biological Laboratory of Amherst College, Amherst.

[2] Plough, H. H., and M. B. Strauss, *J. Gen. Physiol.*, 1923–24, vi, 167.

[3] This mutation was found to be identical with one, designated as Brown-II, which has been isolated from the Columbia University cultures of the Sweden stock. It is an allelomorph of the Brown described by G. Waaler, *Hereditas*, 1921, ii, 391, located at 105, in the second chromosome. Brown-II is quite similar to Purple, and the double recessive is indistinguishable from either Brown-II or Purple. In older flies, whereas Purple seems to fade toward the wild eye color, Brown-II is accentuated.

The "C" line produced its offspring at the usual time, but it had progressed another generation before a fertile pair could be secured from the "A" line, and both of these developed one generation further before the "B" line yielded a pair of fertile progeny. That the climatic changes caused an effect on the germ plasm at this point seems less likely than that there already was a genetic difference between the lines as a result of the inbreeding which manifested itself for the first time under the less favorable environmental conditions.

TABLE I

NUMBER OF OFFSPRING HATCHING FROM PAIR MATINGS IN THE UNITED STATES

Date of counts	Line and generation inbred	Bottle number			Average number of progeny per fertile bottle
		1	2	3	
1923					
Oct. 15–25	A–17	86	67	16	56
	B–16	34	21	13	23
	C–18	212	208	183	201
Oct. 28–Nov. 6	A–18	80	7	2	30
	B–17	10	4	*	7
	C–19	184	183	177	181
Nov. 8–18	A–19	138	114	48	100
	B–18	63	37	34	45
	C–20	227	209	199	212
Nov. 18–28	A–20	57	16	*	36
	B–19	23	10	6	13
	C–21	213	211	177	200
Nov. 29–Dec. 8	A–21	204	39	*	121
	B–20	8	7	6	7
	C–22	256	213	208	226
Dec. 13–23	A–22	177	120	69	122
	B–21	30	7	6	14
	C–23	305	262	254	273
1924					
Jan. 30–Feb. 7	A–26	70	*	*	70
	B–25	17	6	2	8
	C–27	199	187	181	189
Feb. 10–20	A–27	223	147	89	153
	B–26	37	8	*	22
	C–28	237	227	203	222
Summary of	A	total	progeny	1769	88.5
eight gener.	B	"	"	389	17.6
	C	"	"	5115	213.1

Upon the return of the cultures to Amherst they were kept in an electrically controlled incubator at 24° C. in order to eliminate as far as possible environmental changes during the tests. The first determinations made were on fertility as measured by the number of progeny hatching from each pair mating in ten days. The progeny of three pairs of flies were counted in each of the inbred lines. In a set of counts made on eight different generations, spread over five months, and involving over 7,000 flies, it was found that the "C" line gave an average of 213.1 flies per mating, the "A" line 88.5, and the "B" line 17.6. The results of the individual counts are summarized in Table I. The fact that the "C" line, carrying the eye-mutant Brown-II, developed many more imagos than either of the other two lines is interesting in connection with the work of Gonzalez,[4] which indicated that the eye-mutant Purple is connected with a large number of offspring.

A further test of the differences between the lines was offered by egg counts. The method of making the counts was one used by Plough, to whom I may at this point express my indebtedness for his suggestions in all the work. The ordinary banana agar was poured in a thin layer on microscopic slides for about half their length and allowed to set with a plate of glass resting on the agar, thereby smoothing the surface. These were placed in the usual fly bottles with a piece of toweling to absorb moisture in the bottom and the flies introduced. The slides were changed daily, and the eggs laid on the agar counted under a binocular microscope. Following this, the slides were placed in a bottle containing food and allowed to hatch.

The "C" line laid the most eggs, five times as many as the "A" line and six times the "B" line. Although the two latter lines were not very different in the number of eggs laid, 70 per cent. of the "A" line eggs developed into imagos, while only 25 per cent. of the "B" line did so. The "C" line gave about 40 per cent. development, the smallness being possibly accounted for by the crowding of larvae on the slide before they were large enough to reach the other food. The counts are shown in Table II.

Since these results suggest that the difference in numbers of offspring between the lines depends on the number of eggs laid,

[4] Gonzalez, B. M., AMER. NATUR., 1923, lvii, 289.

TABLE II

EGGS LAID IN PAIR MATINGS AND IMAGOS HATCHING IN INBRED LINES

Line and generation	Eggs in ten days from bottle number		Total eggs	Total imagos hatching	Percentage development
	1	2			
A–23	113	18	131	93	70.9%
B–22	59	46	105	27	25.7%
C–24	324	299	623	242	38.8%

one would expect that in first generation crosses the number of progeny would depend on the female line. Castle in his work had found similar results with crosses between lines.[5] To check this up crosses were made between the three lines reciprocally, the results being summarized in Table III. In all six crosses the number of offspring is seen to be approximately the same as for the pure line from which the females were secured. The slight favorable effect of using foreign males, though hardly large enough to be significant, is in line with Hyde's results.[6] How-

TABLE III

NUMBER OF PROGENY PER PAIR MATING IN FIRST GENERATION CROSSES

Line and generation ♀ × ♂	Bottle number			Average number of progeny per fertile bottle
	1	2		
A–25 × B–24	134	112		123
A–25 × C–26	182	2		92
B–24 × C–26	27	3		15
B–24 × A–25	*	*		*
C–26 × A–25	225	225		225
C–26 × B–24	220	201		210
	1	2	3	
A–25 × A–25	70	*	*	70
B–24 × B–24	17	6	2	8
C–26 × C–26	199	187	181	189

ever, in the second generation of the crosses shown in Table IV, the average progeny per pair was considerably higher than any

[5] Castle *et al.*, *Proc.* Am. Acad. Arts and Sci., 1906, xli, 731.
[6] Hyde, R. R., *J. Exp. Zool.*, 1914, xvii, 173.

of the pure line averages, similar to the results of both Castle and Hyde (*loc. cit.*). This suggests that the factors which had depressed the number of offspring in the pure lines were dissimilar and thus the restoration of the heterozygotic state brought the totals beyond those of any of the pure lines.

TABLE IV

PROGENY IN SECOND GENERATION OF CROSSES

Grandparents ♀ × ♂	Bottle number			Average number of progeny per fertile pair
	1	2	3	
A–25 × B–24	293	221	213	242
A–25 × C–26	349	339	271	319
B–24 × C–26	367	321	252	313
B–24 × A–25	no F_1			
C–26 × A–25[7]				
C–26 × B–24	330	289	249	289
A–25 × A–25	223	147	89	153
B–24 × B–24	37	8	*	22
C–26 × C–26	237	227	203	222

During the same period that the control temperature counts were being carried on, tests were made of the relative ability of the lines to tolerate high temperatures. Four sets of tests, involving at least three bottles per line in each test, at temperatures ranging from 30° C. to 32.5° C. showed the three inbred lines unable to produce any second generation offspring, with the exception of a single bottle in the "C" line which just gave enough offspring to carry the line for five generations, one bottle a generation, before being discontinued. In the last test, at 30° C., the Sweden stock and the crossed lines were also tested, but likewise failed after the first generation.

A fifth test, however, made at 29.5° C., produced second generation offspring in the "C" line, the three crossed lines, and the Sweden stock. Two of the crossed lines and the Sweden stock were further able to produce a third generation. This indicates that the same type of genetic differences existed in these lines, all descended from the Sweden strain, as had been noted to exist

[7] An accident prevented any counts being made, but the reciprocal cross of this as well as the reciprocal cross in the case where no first generation hatched is illustrative.

between different strains in the former work. In the first generation hatching at this temperature, the numbers of offspring, shown in Table V, though not as large, bear the same relation to each other as in the control tests.

TABLE V

DIFFERENCE IN TOLERATION OF TEMPERATURE OF 29.5° C.

Line and generation	Bottle number		Average number per fertile bottle	F_1	F_2
	1	2			
A–29	65	20	42	failed	
B–28	6	*	6	failed	
C–30	74	67	70	bred	failed
Sweden	88	81	84	bred	bred
AB cross	132	111	121	bred	bred
BC cross	173	121	147	bred	bred
AC cross	147	30	88	bred	failed

SUMMARY

(1) By inbreeding for from fifteen to thirty generations, three lines of Drosophila were secured from an original pair of wild stock flies which showed clear differences in fertility as measured by offspring produced.

(2) The numbers of eggs laid by females of each of these lines showed similar differentiations.

(3) By crosses between the lines it was shown that the number of first generation progeny depended chiefly upon the female (presumably upon the number of eggs laid) and that the second generation progeny were more numerous than any of the parent line averages, owing to the heterozygotic condition of factors controlling offspring number.

(4) Tests made at 29.5° C. showed that only the original wild stock and two of the crossed lines could breed at that temperature for more than two generations, and that only the two weakest of the inbred lines could not survive more than one generation.

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